

**Behavioral Endocrinology and Reproductive Strategies in
Wild Female Geladas (*Theropithecus gelada*)**

by

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For my parents, Deb and Dave Roberts

You showed me the world and taught me to explore it,
I couldn't have done this without you.

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Abstract

Sexually selected infanticide by males often arises when a new dominant male enters a group that contains unrelated dependent offspring. Under such circumstances, infant deaths hasten their mothers' return to fertility and translate to male reproductive benefits. In contrast, infanticide poses a salient threat to female reproductive fitness. In response to this threat, females often develop counterstrategies to infanticide such as producing deceptive signs of fertility, prematurely weaning dependent young, forming defensive alliances, or spontaneously aborting a pregnancy. Recent evidence from wild geladas (*Theropithecus gelada*), a close relative of baboons, suggests that infanticide following male takeovers is a substantial threat for females with dependent offspring. The research described here explores the physiology and function of female response to the threat of infanticide in a wild population of geladas in the Simien Mountain National Park of Ethiopia.

This study has three major components. First, we used fecal estrogen metabolites, demographic data, and sexual swellings to establish estimates for life history stages as well as hormonal, behavioral, and sexual swelling profiles across the reproductive stages. These were then used as baseline for investigating the function and potential mechanisms of counterstrategies. Second, we found that demographic factors can mediate infanticide risk for females and found that, following takeovers, female physiological stress response reflects this differential risk. Lastly, we investigated female counterstrategies to infanticide in pregnant and lactating female geladas. We first found unambiguous physiological evidence of spontaneous

miscarriages in geladas and illustrated a fitness advantage to females that employed this strategy. In addition, we found demographic and physiological evidence of deceptive fertility following takeovers in lactating female geladas. Together, these results comprise the most complete study of the function and underlying mechanisms (i.e., hormones and behavior) of female reproductive strategies in primates.

Chapter One

Female counterstrategies to sexually selected male infanticide

Introduction

Intersexual conflict arises from a divergence of interests between males and females (Parker, 1979; Chapman, Arnqvist, Bangham, & Rowe, 2003). In sexually reproducing species, one sex, usually the female, is obligated to invest more in any given reproductive event than the other sex, usually the male. This investment begins with gamete production and continues throughout parental care (Trivers, 1972). Investment in offspring can be particularly unequal in mammals compared to other taxa due to the obligate constraints of gestation and lactation. Conversely, males may only be obligated to produce sperm, find a female, and copulate with her. As a result, female reproduction is limited by time and resources whereas male reproduction is limited by the number of females to which he has access (e.g., Bateman, 1948). Consequently, each sex may develop reproductive strategies to reap the highest individual reproductive success from interactions with partners that have conflicting interests. Females generally benefit from being selective and only reproducing with the highest quality mates available whereas males benefit from reproducing with as many females as fast as possible (Trivers, 1972). Perhaps the most extreme, and certainly the most controversial, of male reproductive strategies is infanticide.

Despite the rarity of male infanticide in any given species, it is a widespread reproductive strategy seen across mammalian taxa (reviewed by Ebensperger, 1998). It occurs in invertebrates (e.g., burying beetles, *Nicrophorus orbicollis*, Trumbo, 2006) and birds (e.g., wattled jacanas, *Jacana jacana*, Emlen, Demong, & Emlen, 1989), but appears to be most common in mammals (Ebensperger, 1998). Within mammals, male infanticide has been documented in rodents (e.g., house mice, *Mus musculus*, Labov, Huck, Elwood, & Brooks, 1985; collared lemmings, *Dicrostonyx groenlandicus*, Mallory & Brooks, 1978; black-tailed prairie dogs, *Cynomys ludovicianus*, Hoogland, 1985), carnivores (e.g., grizzly bears, *Ursus arctos*, Dean, Darling, & Lierhaus, 1986; lions, *Panthera leo*, Bertram, 1975), perissodactyls (e.g., feral horses, *Equus caballus*, Cameron, Linklater, Stafford, & Minot, 2003; plains zebras, *Equus burchelli*, Pluhacek, Bartos, & Vichova, 2006), cetaceans (e.g., bottlenose dolphins, *Tursiops truncatus*, Patterson et al., 1998), artiodactyls (e.g., hippopotamuses, *Hippopotamus amphibious*, Lewison, 1998), chiropterans (e.g., white-throated round-eared bat, *Lophostoma silvicolium*, Knoernschild, Ueberschaer, Helbig, & Kalko, 2011), and primates (e.g., hanuman langurs, *Presbytis entellus*, Sugiyama, 1965; chimpanzees, *Pan troglodytes*, Goodall, 1977; red howlers, *Alouatta seniculus*, Agoramoorthy & Rudran, 1995). The majority of cases of male infanticide in mammals can be explained by the sexual selection hypothesis laid out by Hrdy (1979), which explains male infanticide as a means to increase male reproductive success (Ebensperger, 1998; Agrell, Wolff, & Ylönen, 1998; van Schaik & Janson, 2000). Under this hypothesis, male infanticide is defined by three factors; (1) a male will kill only unrelated offspring; (2) the death of offspring will hasten their mothers' return to conceptive cycles; and (3) the male will enhance his reproductive success by both increasing his chances of siring the mothers' next offspring and accelerating the onset of that next reproductive event (Hrdy, 1979; Hausfater & Hrdy, 1984).

Among mammalian species, the prevalence of sexually selected male infanticide (hereafter, infanticide) increases with the ratio between the periods of lactation and gestation (van Schaik, 2000a). It is argued that, as the length of lactation increases relative to the length of gestation, the more time a male can potentially gain by accelerating a female's next birth (sired by the infanticidal male). Consequently, two separate life history pathways result in a high prevalence of infanticide; (1) species that produce highly altricial young (e.g., rodents); and (2) species that produce precocial, but very slow-growing, young (e.g., primates, van Schaik, 2000a). Indeed, infanticide is most studied and appears most prevalent in primates (van Schaik & Janson, 2000).

Within primates, infanticide is predicted by high rates of male replacement, when a new male becomes either the only breeding male or the dominant breeding male in a social group ("takeovers"). In polygynous species (e.g., mountain gorillas, *Gorilla gorilla beringei*, Fossey, 1984; redtail monkeys, *Cercopithecus ascanius*, Struhsaker, 1977), infanticide commonly occurs immediately following male takeovers. Particularly in cases where a new male enters a group as the only breeding male, paternity certainty is high and the new male is at no risk of killing his own offspring. In multimale mating groups (e.g., anubis baboons, *Papio anubis*, Struhsaker & Leland, 1987; red colobus, *Colobus badius*, Struhsaker & Leland, 1985), the immigration of a new male or the ascent of a lower ranking male to the dominant breeding position can result in infanticide. Under these circumstances, it has been suggested that the male uses his individual mating history to evaluate infant paternity (Hrdy, 1979; Borries, Launhardt, Epplen, Epplen, & Winkler, 1999; van Schaik, 2000b). The number of males in a group can also affect the incidence of infanticide such that as the number of males increases, the incidence of infanticide decreases. Three factors may lead to this pattern (van Schaik, 2000b). First, if polyandrous mating is

occurring, paternity certainty is much lower than in polygynous groups; thus the risk of killing one's own infants is suspected to be high enough to suppress the incentive to commit infanticide. Second, the presence of males that are potential fathers would increase the risk for physical aggression towards the infanticidal male. Third, the presence of more males increases competition for matings, thus decreasing the chances that the infanticidal male will in fact father the next offspring with the female (van Schaik, 2000b).

Counterstrategies to male infanticide among female mammals

Given that mammalian female reproduction is limited by the time and resources required for each reproductive event, each event represents a considerable portion of a female's lifetime reproductive fitness. It is therefore not surprising that a number of counterstrategies to infanticide have developed across mammalian species. Here, I include all known counterstrategies to allow comparison among them. I list and describe when and how each might be used to protect female reproductive effort and provide example species in which each counterstrategy has been documented. Counterstrategies can work in two main ways, they can either prevent the infanticide or they can mitigate the loss of the infant. They can also be used during three stages female reproduction: pre-pregnancy, pregnancy, and lactation. Presumably, the potential cost of losing the infant increases across these stages because the mother's energetic investment increases over time. I have included a table that summarizes the time "sink" for each counterstrategy and whether it is a preventative or mitigative strategy to facilitate comparisons (Table 1.1).

Mating with the dominant male

Females reproduce with the dominant male and then remain with the male (or on his territory) throughout lactation or even across multiple pregnancies/litters to continuously receive

protection from that male (described by Agrell et al., 1998). By electing to mate with the best defenders, females ensure the highest chances of their offspring surviving potentially infanticidal intruders. This is a preventative counterstrategy that is used prior to the birth of the potentially at-risk infant. It is used pre-pregnancy, at the time of copulation, and is considered a cheap strategy. Because this is a preventative counterstrategy, it is difficult to find definitive examples of how effective it is in the wild. Indirectly, as evidenced in rodent species, females will choose to mate with the dominant male when given the choice (Horne & Ylönen, 1996) and this can translate to higher infant survival because dominant males are known to be more infanticidal than subordinates (e.g., Vom Saal & Howard, 1982).

Promiscuity

Also known as paternity confusion, promiscuity is another counterstrategy to be used pre-pregnancy, at or around the time of conception in groups with multimale mating systems. By mating with multiple males the female can decrease paternity certainty and therefore decrease the chances that a male will risk killing his own offspring (reviewed in Agrell et al., 1998; Wolff & Macdonald, 2004). Promiscuity has been documented as a preventative counterstrategy in species of primates, lagomorphs, rodents, carnivores, cetaceans, perissodactyls, and artiodactyls that have male infanticide (van Schaik, 2000b). An extreme case is found in hanuman langurs; females will leave their groups to mate with males in all male groups, i.e., males that could potentially enter their group and become infanticidal. This form of the counterstrategy is thought to confuse paternity well before the threat of infanticide exists (Hrdy, 1977).

Deceptive fertility

Also known as “pseudo-estrus” (Hrdy, 1974, 1979), this is perhaps the “cheapest” preventative counterstrategy that can be used during either pregnancy or lactation. A female

producing deceptive fertility will produce all outward signs of fertility in the absence of ovulation. For example, a lactating female langur will solicit copulations from a potentially infanticidal male but continue to nurse her offspring. Pregnant females may also choose this route and mate with the new male while still pregnant to confuse paternity once the infant is born (e.g., Ramirez-Llorens, Di Bitetti, Baldovino, & Janson, 2008). It is important to note that, because it is occurring outside of ovulation, deceptive fertility is only available to species in which (1) female production of reproductive behavior is flexible and not strictly limited to the periovulatory period as in many rodent species (Hrdy, 1979), and (2) males are not able to detect ovulation (e.g., Fuertbauer, Heistermann, Schuelke, & Ostner, 2011). Mating has been reported to prevent infanticide during lactation in several primate (e.g., hanuman langurs, Hrdy, 1974; gorillas, *Gorilla gorilla*, Watts, 1989) and rodent species (e.g., Mongolian gerbils, *Meriones unguiculatus*, Elwood, 1980; house mice, Labov, 1980), but thus far there is no physiological evidence that such mating is deceptive (i.e., that ovulation is not occurring).

A more extreme version of this counterstrategy includes the production of deceptive swellings, a strategy that has been observed in baboons (*Papio hamadryas*, Zinner & Deschner, 2000) and chimpanzees (Wallis, 1982). Exaggerated sexual swellings occur in only about 10% of all primate species (Dunbar, 2001) and are limited to Old World monkeys and apes (Nunn, 1999). In species with exaggerated sexual swellings, females can potentially produce deceptive swellings and the corresponding mating behavior to allow new males the opportunity to mate with them. In effect, this has two adaptive benefits; (1) the act of copulation decreases the male's incentive to kill the female's offspring; and (2) females may increase the probability of their infants' survival while avoiding the costs of being simultaneously pregnant and lactating. Mechanistically, the pathway may be more complicated. Evidence suggests that sexual swellings

are reliant upon circulating estrogen related to fertile cycles (e.g., Gesquiere, Wango, Alberts, & Altmann, 2007). Although lactation among mammals is generally characterized by very low ovarian hormone levels, pregnancy is characterized by steadily increasing levels of both progesterone and estrogen. However, pregnancy is sensitive to slight perturbations in hormones; estrogen agonists or hypersensitivity to estrogen during pregnancy can lead to miscarriage (e.g., Itsekson, Seidman, Zolti, Lazarov, & Carp, 2007). Due to the heavy energetic cost of lactation (Wade & Schneider, 1992), it could be disrupted if ovulation is actually stimulated. The hormonal changes associated with the resumption of ovulation would lead to the cessation of milk production as energy is redirected to the next potential reproductive event.

Maternal aggression

The risk of infanticide is thought to be the driving force behind an increase in aggressive behavior in females during late gestation and lactation across mammals (e.g., Svare, 1977; Parmigiani, Palanza, & Brain, 1989). As a preventative counterstrategy to infanticide, maternal aggression can be used during lactation to protect their infants. There are reports in multiple species of lactating females increasing aggression towards potentially infanticidal males (e.g., reviewed in Ebensperger 1998). As a counterstrategy, maternal aggression has been seen to be successful in protecting against infanticide in some species of rodents (e.g., desert woodrats, *Neotoma lepida*, Fleming, 1979; meadow voles, *Microtus pennsylvanicus*, Storey & Snow, 1987), while only delaying infanticide in others (collared lemmings, Mallory & Brooks, 1980; house mice, Palanza, Parmigiani, & Saal, 1994). Outside of rodents, and particularly in species where sexual dimorphism is high, increased maternal aggression has not been found to be a successful counterstrategy to infanticide (hanuman langurs, Mohnot, 1971; gorillas, Watts, 1989; fur seals, *Arctocephalus australis*, Harcourt, 1992).

An extension of maternal aggression as a counterstrategy to infanticide is territoriality. Female defense of their territory or nest during pregnancy and lactation can be a preventative counterstrategy to infanticide. There is some supportive evidence of this counterstrategy in rodents as nest defense increases in pregnancy and lactation alongside aggression (reviewed in Ebensperger 1998). However, in some species, much territoriality seems to be misdirected towards the non-infanticidal females (e.g., arctic ground squirrels, *Spermophilus parryii*, Mclean, 1982). Territoriality is not, however, a potential counterstrategy for primates due to their high level of movement and lack of permanent sleeping sites (Hrdy 1979).

Defensive alliances (group defense)

Defensive alliances are associations between individuals that provide allies in agonistic conflicts (Smuts & Smuts, 1993). Such alliances can be used to protect offspring from infanticidal males (e.g., Hrdy, 1977; reviewed in Ebensperger, 1998). Defensive alliances are a preventative counterstrategy seen in primates and carnivores. Most examples of such alliances in primate species (e.g., hanuman langurs, red howler monkeys, and capuchins) are only successful in delaying infanticide, not preventing it (reviewed in Ebensperger, 1998). Coalitions between female lions can successfully protect cubs, but can lead to serious wounds and, in some cases, the death of a female (Packer & Pusey, 1983).

An alternative view on defensive alliances is found at the evolutionary level. It is suggested that infanticide is a strong selective pressure for male-female bonds that exist outside of estrus (reviewed by Palombit, 1999). If male reproductive success is at risk due to the threat of infanticide, this can select for monogamy in which a male protects the female and their offspring (Wolff & Macdonald, 2004). In the case of monogamy, a female will receive all protective benefits from the male.

Pregnancy termination (Bruce effect)

First described in laboratory mice in the 1950s, the Bruce effect is the spontaneous abortion (i.e., miscarriage) of a fetus (Bruce, 1959). It has been documented in a number of rodents in the laboratory (reviewed in Labov, 1981) and in feral as well as domestic horses (Berger, 1983; Bartos, Bartosova, Pluhacek, & Sindelarova, 2011, respectively). Although it has been suggested as possibly occurring in a number of wild populations (e.g., lions, Bertram, 1975; hanuman langurs, Agoramoorthy, Mohnot, Sommer, & Srivastava, 1988), there is no direct evidence to date of this counterstrategy being used in the wild. This could be due to the high cost of this counterstrategy. Pregnancy termination consists of losing much, if not all, energy expended to date within a reproductive event. This would be costly to the female in any species, but it would be relatively more costly for a primate to use this strategy than in rodents or lions because primates have greater investment per infant compared to the other species (Hrdy, 1979; van Noordwijk & van Schaik, 2000). Despite the cost, pregnancy termination is thought to prevent the unnecessary loss of further investment in offspring that will eventually be killed (e.g., Hrdy, 1979), making it a mitigative counterstrategy.

Avoidance of the infanticidal male

Females could leave their social groups or territories altogether to avoid potentially infanticidal males (e.g., Hanuman langurs, Hrdy, 1977; blue monkeys, *Cercopithecus mitus stuhlmanni*, Butynski, 1982; lions, Packer & Pusey, 1983; arctic ground squirrels, Mclean, 1983). This is a mitigative counterstrategy that is used during lactation because the infanticidal male may no longer be a threat, but there is potential for other costs. Female African lions (have been observed to leave their pride with their cubs following a takeover (Packer and Pusey, 1983). This counterstrategy has also been documented to successfully protect young in primates (e.g.,

purple-faced leaf monkeys, *Presbytis senex senex*, Rudran, 1973) and rodents (e.g., alpine marmots, *Marmota marmota*, Coulon, Graziani, Allaine, Bel, & Poudroux, 1995). However, very few data exist on the costs versus benefits of leaving the group or territory. Possible costs include: loss of familiar range or social partners, loss of access to or knowledge of resources, increased predation risk, conspecific aggression associated with trying to enter a new social group, and delay in next reproductive event (Sterck & Korstjens, 2000).

Simultaneous pregnancy and lactation

It may be possible for females that have postpartum mating to actually return to conceptive cycles as opposed to deceptively cycling. This counterstrategy, which is expected to be costly, would be mitigative in that reproducing with the male during lactation should alleviate the male's incentive to kill the current offspring. However, it would be so energetically expensive that it may compromise the success of both reproductive events. Indeed, some species routinely mate during the postpartum period and bear the cost of pregnancy and lactation simultaneously (e.g., white-toothed shrew, *Crocidura russula monacha*, Mover, Ar, & Hellwing, 1989). However, infanticide is likely not seen in species that have postpartum mating (van Schaik & Kappeler, 1997). Likewise, in species that have little to no paternal care, it is unlikely that a lactating female could return to conceptive cycles while continuing to lactate and later simultaneously care for infants at different ages (van Schaik & Kappeler 1997).

Premature weaning

The abandoning or early weaning of young infants is potentially a very costly mitigative counterstrategy. Here, lactating females risk losing all of the time and energy invested throughout pregnancy and lactation up until the premature weaning if the infant is killed or dies. This counterstrategy has only been reported in cases when the infant is old enough to survive on

their own. Lactating hamadryas baboons have been reported to avoid infanticide by shortening their lactational period in response to the arrival of a new male (Colmenares & Gomendio, 1988). There was also a case in which a female ursine colobus mother stopped nursing and associating with her infant following male immigration and this was successful in preventing any further attacks on the infant (Teichroeb & Sicotte, 2008).

Geladas

Geladas (*Theropithecus gelada*) – a close relative of baboons (*Papio* spp.) – are endemic to the highlands of Ethiopia and the sole surviving species of a genus once found throughout sub-Saharan Africa (Jolly, 1972). Although geladas are often mistakenly called “baboons”, genetic evidence places them as a sister taxon to *Papio* (Page, Chiu, & Goodman, 1999). Their range is limited to the highlands of Ethiopia between 1500 and 5000m above sea level (a.s.l.) where protein rich afroalpine grasses make up the vast majority of their diet (Iwamoto, 1993). Although geladas were first “discovered” in the 1800s by German naturalist Rüppell (1835, as cited in Crook, 1966), their remote location has led to them becoming one of the most understudied terrestrial primates in the world.

The basic organization and demography of the gelada social system were outlined in a number of short-term, observational studies conducted in the late 1900s. They have recently been revisited using non-invasive genetic techniques to provide a clear understanding of the gelada social system. Geladas live in multi-level societies consisting of nested reproductive units within larger groups (Dunbar & Dunbar, 1975; Dunbar, 1984; Kawai, Ohsawa, Mori, & Dunbar, 1983; Snyder-Mackler, Beehner, & Bergman, 2012a). Almost all social interactions occur at within the basic groupings: reproductive units (hereafter, units) or all-male groups (AMGs). A unit is made up of 1-12 adult females, a leader male that has almost exclusive reproductive access to the

females, and variable numbers of offspring (e.g., Crook, 1966; Dunbar and Dunbar, 1975).

Leader males attend closely to their females, but never attempt to steal or mate with females in other units (Dunbar, 1984). In approximately one third of units, additional males, termed follower males, live and socialize within the unit, but are subordinate to the leader and rarely mate with females (Dunbar, 1984; Snyder-Mackler, Alberts, & Bergman, 2012b). Followers can either be the deposed leader male that remains in the group or another outside male that enters the unit during the takeover. Recent genetic analyses indicate that 100% of infant paternity is within the unit. In the cases of multiple males in the group, all offspring are fathered by either the leaders (83%) or followers (17%) of their unit (Snyder-Mackler et al., 2012b).

Geladas are a female-philopatric species; females remain with their female relatives in their natal units (le Roux, Beehner, & Bergman, 2011), while males disperse (Dunbar and Dunbar, 1975). Following dispersal, males congregate in AMGs, generally comprised of 2-15 males ranging in age from subadult to old (Dunbar, 1993; Kawai et al., 1983, Pappano & Beehner, 2012). Males remain in AMGs until they are able to successfully challenge and take over a unit of their own (hereafter, a “takeover”) (Beehner & Bergman, 2008). Multiple units and any associated AMGs that share a common home range more than 50% of the time are called bands. Bands that come together for sleeping or foraging are called a community (Snyder-Mackler et al., 2012a, Kawai et al., 1983). While membership in units is stable in between male takeovers, fission and fusion are common at the levels of band and community, even within a single day (Kawai et al., 1983).

Female geladas, like many Old World monkeys, produce sexual swellings around the time of ovulation (Dunbar & Dunbar, 1974). However, unlike other monkeys, gelada sexual swellings are situated around a patch of exposed skin on the chest. This skin prominently

changes color and becomes outlined in swollen, bead-like vesicles during the periovulatory period (Dunbar & Dunbar, 1974) and also undergoes changes in color during pregnancy and lactation (McCann, 1995). Geladas also have perineal swellings, but these swellings are much smaller and less variable in color and size than those of other species.

Infanticide in geladas

For decades infanticide was not observed in wild geladas (Dunbar & Dunbar 1975). It was thought not to occur in geladas because females mated with the new males soon after takeovers to inhibit males from committing infanticide (Dunbar, 1980, Mori & Dunbar, 1985). Upon closer inspection, and with new evidence, it is clear that the gelada social and reproductive systems make them highly vulnerable to infanticide. This is particularly clear when examined in the context of the 3 requirements of sexually selected male infanticide (Hrdy, 1979): (1) *A male will kill only unrelated offspring*. Paternity is 100% within unit (leader and follower), any entering male will not have fathered any of the offspring. (2) *The death of offspring will hasten their mothers' return to conceptive cycles*. Although it is only anecdotal, evidence suggests that infanticide can shorten the time to cycling for mothers (Mori, Iwamoto, & Bekele, 1997). (3) *The male will enhance his reproductive success by both increasing his chances of siring the mothers' next offspring and accelerating the onset of that next reproductive event*. Geladas have young that are dependent for a long period of time (i.e., high lactation to gestation ratio) and have no distinct breeding season. Therefore, reproductive success for gelada males is affected by their tenure as a unit leader male and the size of their unit; longer tenures and larger units lead to higher reproductive success (Dunbar, 1984). As a result, males entering a unit stand to substantially benefit by accelerating the start of their reproductive timeline as leader (i.e., reproducing with every unit female as soon as possible).

For the last several decades anecdotal evidence on wild geladas has been mounting to suggest that infanticide following a male takeover may be a real threat for females with dependent offspring (Mori et al., 1997, Mori, Belay, & Iwamoto, 2003). Recent work on geladas indicates that infanticide is the leading cause of infant mortality in a population of wild geladas living in the Simien Mountains National Park of Ethiopia (Beehner & Bergman, 2008). In response to this salient threat, some pregnant females may spontaneously abort or produce deceptive swellings to mimic the signs of fertility (Mori & Dunbar, 1985; Beehner, unpublished data). Indeed, such reproductive tactics have been reported in other primate species as a possible way to conserve or protect costly reproductive effort (e.g., Wallis, 1982, Zinner & Deschner, 2000). However, the physiological mechanisms behind such reproductive strategies have never been explored in primates.

Geladas provide an ideal system for studying female counterstrategies to infanticide following takeovers because they have the potential to engage in many of counterstrategies seen in other species. Deceptive swellings could be produced by females (i.e., swellings in the absence of ovulation). Although geladas are thought to produce sexual swellings only around the time of ovulation, they have been documented to copulate throughout the cycle (Dunbar & Dunbar, 1974). The flexibility in their mating behavior may indicate more flexibility in the hormonal determination of their swellings. Maternal aggression could be used by the mother to physically protect her infant, increasing the risk to the male if he attempts infanticide. Defensive alliances would be possible with the deposed leader male or other females in the polygynous reproductive units. Here females would recruit their allies to physically protect their infant. A Bruce effect (or spontaneous pregnancy loss; e.g., house mice; Bruce, 1959), although never conclusively recorded outside of laboratory rodents, may be seen in females that are pregnant at

the time of takeovers to avoid eventual infanticide. Premature weaning of infants may occur such that the infant would remain with the group and be able to survive even if it is no longer nursing.

Geladas are unlikely to produce other counterstrategies, mainly due to several constraints imposed by their social system. Neither of the pre-pregnancy counterstrategies, mating with the dominant male and promiscuity, would be options for them due to unit structure. Although females do seem to have some role in choosing the leader male (Dunbar & Dunbar, 1975), there is only one leader male in a unit at a time. Therefore females do not have the option to choose a dominant male over a subordinate to protect her young from the subordinate. In cases where there is a follower that fathered offspring in a unit, there is no record of a current leader male committing infanticide. Similarly, promiscuity would not benefit the female because females have only been documented mating with the leader and follower males in their units and there is no evidence of a follower male ever taking over a unit. Avoidance is unlikely as female emigration is all but unheard of in the female-centered gelada social system (Dunbar, 1984). Simultaneous pregnancy and lactation is highly unlikely due to the lack of evidence in any other primate and the energetic costs involved.

The overall goal of this dissertation is to explore female counterstrategies to infanticide in wild geladas. In addition to the potential for geladas to produce multiple counterstrategies, their social system allows for sufficient data collection in a short amount of time because: (1) their complex, multilevel society allows for the simultaneous monitoring of over 20 social groups and (2) they have a high rate of takeovers. In combination with this favorable system, the availability of non-invasive hormone collection methods allow the addition of a physiological approach to this study. I aimed to document each female's hormonal response to a male takeover, even when it did not actually result in infanticide. Thus, the mechanisms (i.e., hormones) that underlie

observed behavioral responses can yield a richer understanding of the adaptive significance of any female counterstrategy to infanticide.

Study Site and Subjects

The Simien Mountains National Park (SMNP) is located in the Gondar Province of northern Ethiopia. It was established in 1969 in an effort to protect the endangered Walia ibex (*Capra ibex walie*). It has since become a UNESCO World Heritage Site (1978) and, in addition to the Walia ibex, protects populations of geladas and the endangered Ethiopian wolf. The park covers approximately 169km², consists of a series of highland plateaus and the surrounding lowlands, and has an altitudinal range of 1700-4500m a.s.l.. The park and surrounding area is heavily inhabited by humans. Crops, mainly barley, are grown around villages throughout the park and there is heavy livestock grazing up to about 4000m.

The research presented here was collected as part of the University of Michigan Gelada Research Project (UMGRP). This research was approved by the University Committee on Use and Care of Animals (UCUCA #09554) at the University of Michigan and adhered to the laws and guidelines of Ethiopia. UMGRP was established in 2006 by Drs. Jacinta Beehner and Thore Bergman. Since it began, UMGRP has monitored a population of wild geladas living in the Sankaber area of the Simien Mountains National Park, Ethiopia (13°15'N, 38° 00'E). A portion of this area is protected by park officials from livestock grazing, cultivation, and collection of resources (e.g., wood). This protection likely makes this population of geladas the least disturbed in the park. Sankaber is made up of a plateau at approximately 3250m in altitude with an 800m sheer cliff to the north and steep slopes into a deep gorge to the south. Geladas climb down the cliffs or slopes at night and range around the top of the plateau during the day. Data presented here were collected between Jan 2006 and Jun 2012.

Questions

Below I describe the four questions addressed in my dissertation research. Each question will be addressed in a subsequent chapter (Two-Five). Finally, in Chapter Six, I will briefly summarize the major findings of my dissertation research and synthesize these findings in the context of suggested future directions. Here, I briefly outline the background, objectives, and hypotheses for each question, where appropriate. Although I was the primary person collecting the data, conducting the analyses, and writing up the results, each subsequent chapter is a collaborative effort with coauthors who (1) collected data, (2) provided assistance in the study design, and/or (3) the interpretation and analysis of the results. Consequently, I use the pronoun “we” throughout the thesis.

1. What are the normal patterns of gelada reproduction?

Although our overall goal is to investigate reproductive counterstrategies to infanticide, we must first close the gap in knowledge of basic reproduction in geladas. By establishing normal reproductive patterns, we provide a baseline for each female reproductive stage (cycling, pregnancy, and lactation) for comparison with female responses when infanticide is a threat. Using this baseline we will then investigate (1) the function and (2) the mechanism of any counterstrategies. In Chapter Two, we set out to establish detailed normative patterns for these two goals: (1) Normal patterns of life history stages will be provided as a means of identifying any benefits to female reproductive success that would indicate the function of any counterstrategies. (2) For identifying mechanism, we provide the profiles of hormones, behavior and sexual swellings of wild female geladas across cycles, pregnancy, and lactation.

2. Do females at risk for infanticide perceive that risk following male takeover?

The stress response is an adaptation that has evolved to aid in the protection of an organism from both social and ecological challenges (Sapolsky, 2002). Recently, Beehner, Bergman, Cheney, Seyfarth, & Whitten (2005) demonstrated that pregnant and lactating, but not cycling, female chacma baboons were stressed by the arrival of a new, and potentially infanticidal, male. The observed physiological response, as measured by an increase in the stress hormone glucocorticoid (GC), suggests that females perceive the threat of infanticide, if (and only if) they are at risk. Given that infanticide in chacma baboons is responsible for almost 60% of infant mortality (Cheney et al., 2004), comparable to the almost 50% seen in geladas (Beehner & Bergman, 2008), we expect to find a similar pattern among lactating, pregnant, and cycling females following takeovers in geladas, with a rise in GCs in lactating and pregnant females, but no rise in cycling females. In Chapter Three, we examine the demographic factors that might mediate infanticide risk for individual females and how well that risk is reflected in female physiological stress response to takeovers (i.e., how well a female perceives her own risk of infanticide). The following two questions focus on the two groups of females that are at risk for infanticide.

3. Do pregnant female geladas use counterstrategies to infanticide following takeovers?

The first group of females that is at risk for infanticide is pregnant females. As discussed above, females that are pregnant when faced with the risk of infanticide have several potential counterstrategies. Overall, they can either continue to put energy into the pregnancy and carry it to term (with a high risk that the infant will be killed), or they can mitigate their losses by ceasing to invest in that reproductive event. If females continue to put effort into the pregnancy, they are expected to use other counterstrategies to prevent infanticide either during pregnancy or after parturition. Here we focus on the period during pregnancy, immediately following male

takeover. There are two potential counterstrategies that females could use during this time: deceptive fertility or a Bruce effect. The cheaper, and perhaps the most likely, behavioral counterstrategy would be deceptive fertility. If this is the case, females would produce sexual swellings and behavior in the absence of ovulation. By deceptively copulating with the new leader male (when they cannot be inseminated by him), females may decrease the male's incentive to kill her offspring after parturition. Alternatively, female geladas may show a Bruce effect and spontaneously lose their pregnancies. This would allow females to mitigate their losses, ceasing to invest in offspring that are very likely to be killed. However, the Bruce effect has never been confirmed in a primate, nor in any species in the wild.

Anecdotal evidence suggests that perhaps both of these counterstrategies are employed by geladas. Mori & Dunbar (1985) reported that most females begin to swell soon after a takeover, but a number of assumed miscarriages have also been reported (Mori & Dunbar, 1985; Beehner & Bergman, 2008). Because a Bruce effect would seem to be more costly than deceptive fertility, our original hypothesis was that pregnant females would use deceptive fertility to avoid eventual infanticide. However, upon examination of the demographic history of our study population we found that there was a significant decrease in births following takeovers. Therefore we changed our hypothesis to be that pregnant female geladas use a Bruce effect as a counterstrategy to infanticide. In Chapter Four, we investigate the mechanism and function of a potential Bruce effect in pregnant females.

4. Do lactating female geladas use counterstrategies to infanticide following takeovers?

The second, and more obvious, group that is at risk for infanticide is lactating females. A wider variety of counterstrategies have been seen in lactating than pregnant females across mammalian taxa, including: deceptive fertility, maternal aggression, defensive alliances, and

premature weaning. Although more difficult in species with sexual swellings, deceptive fertility is an option for geladas. Maternal aggression and defensive alliances are certainly possible. Defensive alliances may be particularly effective if the father of the infant remains in the unit as a follower, in this case both the mother and father have reproductive investment to protect. Alternatively, defensive alliances with high ranking females or coalitions with other lactating females may be used. Premature weaning may also be an available counterstrategy for geladas. Given that these stable groups of individuals are made up of close relatives, a prematurely weaned infant that can feed itself may still receive the protection of the unit and be able to survive.

Again, anecdotal evidence in geladas would suggest that females use deceptive fertility as they begin swelling and mating with the male soon after male takeover (Mori & Dunbar, 1985). Therefore our hypothesis was that lactating females would produce deceptive fertility as a counterstrategy to infanticide. In Chapter Five, we examine the hormones, behavior, and swellings of lactating females in order to test this hypothesis against the other potential counterstrategies.

Table 1.1. Summary of type and timing of female counterstrategies to infanticide.

Type	Timing of Counterstrategy
Preventative	
Mating with the dominant male	Pre-Pregnancy
Promiscuity	Pre-Pregnancy
Deceptive fertility	Pregnancy/Lactation
Maternal aggression	Lactation
Defensive alliances	Lactation
Mitigative	
Pregnancy termination (Bruce effect)	Pregnancy
Avoidance	Pregnancy/Lactation
Simultaneous pregnancy and lactation	Lactation
Premature weaning	Lactation

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Chapter Two

Reproductive parameters of wild female geladas (*Theropithecus gelada*)

Introduction

For any species of mammal, an understanding of reproductive parameters, such as cycle length, gestation length, and interbirth interval, is necessary for exploring its evolution, ecology and social behavior. These basic patterns are utilized in comparative studies across species (e.g., Borries, Koenig, & Winkler, 2001) and populations (e.g., Higham, Warren, Adanu, Umaru, Maclarnon, Sommer, & Ross, 2009), but are also crucial to the study of within-species phenomenon such as adaptive reproductive strategies (e.g., Engelhardt, Hodges, & Heistermann, 2007; Zhao, Borries, & Pan, 2011; Roberts, Lu, Bergman, & Beehner, 2012). Furthermore, detailed knowledge of reproductive parameters is critical for designing and implementing effective management and conservation efforts (e.g., Cockrem, 2005).

Geladas (*Theropithecus gelada*) are a species of Old World Monkey endemic to the highlands of Ethiopia. Although the gelada social system and demography have been examined in a number of studies, female reproductive parameters have yet to be well described in this system. Geladas live in a complex multi-leveled society with as many as 1100 individuals found together (Snyder-Mackler, Beehner, & Bergman, 2012). The majority of social interactions, however, occur only within a small, stable set of individuals known as the “reproductive unit” (hereafter, “unit”). Units are generally comprised of 1 dominant leader male, 1-12 reproductive

females, and their dependent offspring (e.g., Dunbar & Dunbar, 1975). Females within a unit are close kin (Snyder-Mackler, in prep) and remain in their natal unit throughout their lives (le Roux, Beehner, & Bergman, 2011). In contrast, leader males are regularly replaced by non-breeding bachelor males (hereafter, “takeover”) at a fairly high rate of 0.30 takeovers per unit per year (Beehner & Bergman, 2008). Multiple units and any associated bachelor groups (congregations of 2-15 males) that share a common home range more than 50% of the time are called “bands”, and bands that join together for sleeping or foraging throughout the year make up a “community” (Snyder-Mackler et al., 2012, Kawai, Ohsawa, Mori, & Dunbar, 1983). Temporary aggregations of geladas are generically referred to as “herds” and can comprise any mixture of units from one or more bands.

Geladas are an excellent species for comparative work. First, they have several derived characteristics with respect to their close relatives (the *Papio* baboons). In addition to their multi-level social system (relatively rare among cercopithecines: only hamadryas baboons, *Papio hamadryas*, also exhibit a multi-level society, Schreier & Swedell, 2009), geladas have two additional characteristics that make them unique among primates: (1) they are the only graminivorous (i.e., grass-eating) primate (Dunbar, 1983); and, (2) unlike the perineal sexual swellings of some other primates, gelada females have sexual skin swellings on their chest and neck (Matthews, 1956; Alvarez, 1973; Dunbar & Dunbar, 1974). Second, although the reproductive parameters of baboons are well described (e.g., Altmann, Altmann, Hausfater, & McCuskey, 1977; Sigg, Stolba, Abegglen, & Dasser, 1982; Smuts & Nicholson, 1989; Higham et al., 2009), the same measures in geladas are almost entirely unknown for wild populations (and even fairly scanty for captive populations).

Geladas are also an ideal species for examining female reproductive strategies. For example, sexually selected male infanticide, a widespread male reproductive strategy among mammals (e.g., van Schaik & Janson, 2000), was found to be the leading cause of infant mortality in a wild gelada population (Beehner & Bergman, 2008). In response to this threat, sexual conflict theory predicts that females should have one or more strategies to protect their offspring against this threat. Indeed, supporting this prediction, gelada females have at least two “counterstrategies” to male sexually selected infanticide (detailed in subsequent chapters). Importantly, *both* of these counterstrategies involve physiological mechanisms related to female reproduction. Therefore, as a critical first step towards understanding these strategies further, we need to establish baseline female reproductive parameters for wild geladas.

Finally, geladas are in need of improved conservation efforts. Although geladas are categorized as “Least Concern” by the IUCN, their numbers are decreasing (IUCN, 2008). Geladas have a very limited range and are threatened by both habitat destruction and human encroachment. They reside only in areas that include grassy plateaus alongside rocky gorges or cliffs between 1500 and 5000m a.s.l. with most of them living between 2000 and 3000m a.s.l. (Iwamoto, 1993). Although geladas are widespread across the central Ethiopian plateau, the only officially protected population is found in the Simien Mountains National Park in northern Ethiopia (IUCN, 2008). By using this protected population to establish baseline reproductive parameters, we aim to provide knowledge that will be vital to the monitoring of gelada populations and guide the implementation of appropriate conservation action.

To date, the reproductive parameters of female geladas have only been roughly documented in wild populations. Dunbar (1980) used estimated female and infant ages and observational data to calculate each of the following: age at first birth, cycle length, time to

conception, gestation, postpartum amenorrhea, and interbirth interval. However, in addition to using rough estimates for female age, all calculations were based on two 9-month field seasons, separated by two years. The abbreviated timeline of these discontinuous field seasons did not allow for observation of complete reproductive sequences. Further, all reproductive parameters were estimated from observations of behavior and morphology only and not confirmed with ovarian hormones. Here, we build on this foundation using complete life histories from females with known birthdates (to establish maturational milestones) and known ovarian hormone patterns (to establish reproductive parameters). To our knowledge, this dataset represents the most complete and most accurate reproductive data on wild geladas to date.

One study on captive geladas in the Bronx Zoo examined the behavior, sexual swellings and ovarian hormone activity of female geladas in detail (McCann, 1995). This study focused on the distinct phases of the menstrual cycle, documenting the lengths of the follicular and luteal phases. Based on these data, gelada females have approximately 40-day menstrual cycles and 180-day gestation periods (McCann, 1995). The menstrual cycle, as measured by progesterone, includes a 20-day follicular phase and an 18-day luteal phase. However, estrogen, the hormone generally responsible for female reproductive behavior and sexual swellings in primates, has heretofore not been measured in captive or wild geladas.

Here we use fecal estrogen (fE) profiles together with detailed demographic and observational data to establish female reproductive parameters in a group of wild geladas living in the Simien Mountains National Park, Ethiopia. First, we establish maturational milestones related to life history (i.e., age at maturation, age at first birth) as well as basic reproductive parameters (i.e., interbirth interval, cycle length, gestation length, postpartum amenorrhea, and

time to conception). Second, we examine the specific reproductive characteristics (i.e., hormonal, morphological, and behavioral changes) of ovarian cycles, gestation, and lactation.

Methods

Study site and subjects

This research was part of the long-term University of Michigan Gelada Research Project (UMGRP) that has been continuously monitoring a wild population of geladas in the Simien Mountains National Park (13° 15'N, 38° 00'E, elevation: 3250m a.s.l.) since 2006. We combined data from the long-term project (where hormone sampling for females was less detailed) with data from EKR's 2-year thesis project (where hormone sampling for females was more detailed). Specifically, we utilized observational data from 77 females across 18 units (2006-2011) with hormonal data on a subset of these females (63 females in 15 units; see Table 2.1 for a summary of fecal sampling). All UMGRP target animals are individually recognized and fully habituated to observers on foot. All of our methods for this study were non-invasive, and all research was approved by both the Ethiopian Wildlife Conservation Authority and the University of Michigan University Committee for the Use and Care of Animals.

Observational data collection

We monitored females on all days they were encountered for life history events including: infant births, deaths, and takeovers. For the majority of births, we did not observe them directly and therefore assigned an infant birth date as the first morning they were seen. When females that gave birth were not monitored for two or more days (and in the absence of other information such as blood on the mother, a wet infant, a wet umbilical cord), we assigned a birth date corresponding to the midpoint of the missing period. All infants with birth dates estimated over a period longer than 2 weeks were not included in analyses unless otherwise

noted. Deaths were assigned when a target individual was missing from its unit for three consecutive days. Post hoc we then assigned the first date on which it was missing as the date of death. When a new male became the dominant leader of a unit, we recorded this event as a takeover. A unit undergoing a takeover is generally characterized by intense aggression between the incumbent and challenging male(s). This period of instability can last from hours to weeks. For cases in which the challenging male became the new leader male, we assigned the date of takeover as the first day of his challenge. When units were not monitored for two or more days (and in the absence of any other information), we assigned the date of takeover as the first day that the unit was not observed.

We monitored females for reproductive condition on a regular basis using sexual swellings and the presence of an infant. Given the clean patterns of swellings during fertile periods in baboon species (e.g., Gesquiere, Wango, Alberts, & Altmann, 2007; Higham, MacLarnon, Ross, Heistermann, & Semple, 2008), we operated under the assumptions that sexual swellings indicated a “cycling” female and that a disruption in sexual swellings indicated a “pregnant” female (as this and later chapters will illustrate, this was not always true). When females had infants, we scored them as “lactating”.

Female sexual swellings in geladas are much more varied than the typical baboon sexual swelling, but generally form a “necklace” of bead-like vesicles along the edges of a hairless patch of skin on the chest (Figure 2.1). In an effort to quantify all dimensions of this complex trait, we scored sexual swellings using five different measurements: (1) chest color (patch color, not vesicle color), (2) vesicle pattern, (3) turgidity of vesicles, (4) perineal swelling (this is similar to, but much smaller than, perineal swellings observed in other primate taxa), and (5) paracollousal skin color (Figures 2.1-2.2). We scored chest color using a 1-5 color scale with

larger numbers indicating more red (Figure 2.2). Vesicle pattern was separated into 16 distinct categories of “organization” represented by in Figure 2.2. Turgidity (or “fullness” of the vesicles) was scored on a scale from 0-3, with 0 indicating no vesicles and 3 indicating maximally swollen vesicles (Figure 2.1). Perineal swellings were scored only on a presence/absence basis because the amount of hair around these vesicles prevented accurate turgidity scores. Paracollousal skin color was always scored for whether skin was black, red, or mottled (part black and part red). In Jan 2010, we added a second measure, which scored any pink paracollousal skin on a 1-5 point scale (the same as for chest color, as described above).

We also monitored female sexual behavior on a regular basis. We recorded both proceptive and receptive behaviors (Beach, 1976). Proceptive behaviors are those that are considered to function in soliciting the male; for our purposes these included two proceptive behaviors: pre-copulatory calls and presents to the male. Receptive behaviors generally include any behaviors that indicate a female “accepts” a sexual advance from a male (Beach, 1976). For geladas (as in many primate species), receptivity is measured only by the act of copulation (see Table 2.2 for definitions).

As part of the long-term monitoring for the UMGRP, all project personnel collect 15-minute focal animal samples (Altmann, 1974) to record all social behaviors for focal individuals. Although focal observations include all sexual behaviors, we recognize that it is difficult to capture rare behaviors in focal samples alone. Therefore, we supplemented the focal samples with all occurrence records of copulations which included pre-copulatory calls and presents when the observer was able to catch the entire interaction. Note, if sexual behavior was captured in a focal animal sample, it was not also recorded in the all occurrence sampling. Using both sets of behavioral data, we assigned each female a daily 1-0 score for each sexual behavior. A female

was assigned a “1” on days when that behavior was observed and a “0” on days when it was not observed. Because we were primarily interested in whether such behaviors occurred or not (and are not concerned with rates of these behaviors), combining these two sources of data did not pose a problem for our analyses.

Hormone data collection

We extracted steroid hormones from fecal samples collected from known individuals. This method of collection and preservation has been described previously and all hormones discussed herein have been previously validated for use in geladas (Beehner & McCann, 2008; Roberts et al., 2012). To summarize this method, we followed known individuals until they produced fecal samples. Within a few minutes of excretion, we mixed samples thoroughly with a wooden spatula, placed them in a methanol-acetone solution, and homogenized them with a battery-powered vortexer. About 8 hours post-collection, we filtered samples (0.2 μm , PTFE Whatman filter, Whatman, Clifton, NJ), diluted them with water, and loaded them onto a primed octadecylsilane cartridge (Sep-Pak Plus; Waters Associates, Milford, MA). We then washed cartridges with a 0.1% solution of sodium azide (to reduce steroid degradation) and placed them in a sterile Whirl-Pak bag. We allowed the fecal matrix to dry and later obtained the dry weight ($\pm 0.001\text{g}$). We stored all cartridges at subzero temperatures (-10°C) in the field until carried or shipped to the University of Michigan for analysis. Once in the laboratory, we incubated samples at room temperature for one hour and eluted hormones from cartridges with 2.5ml 100% methanol. We then froze all eluted samples (-20°C) until the time of radioimmunoassay (RIA).

For the RIA, we assayed all samples for estrogen metabolites using a commercially available kit (17 β -estradiol Double Antibody RIA kit, MP Biomedicals, see Roberts et al., 2012 for validation). Prior to RIA, all samples were incubated at room temperature for one hour. Then,

an aliquot of each sample was evaporated to dryness under nitrogen. Sample aliquots were determined such that hormone metabolite values were within the range of optimal precision of the assay. Kit protocols were followed except that all reagents were halved from the amount suggested by the manufacturer (a common technique employed by researchers measuring fecal steroids to maximize the use of each kit). Internal controls were run in every assay and consisted of a high (binding at 20%) and a low (binding at 80%) “pool” (a composite of many fecal samples). All standards were run in triplicate, all controls and samples were run in duplicate, and mean concentrations were expressed as ng per dry gram of fecal material (ng/g). The MP Biomedicals E2 antibody is known to have minor cross-reactivities with other estrogen metabolites (estrone: 20%; estriol: 1.5%; estradiol-17 α : 0.7%). Inter- and intra-assay CVs for a low and high sample were 15.42% and 14.24% (N=34) and 8.74% and 14.73% (N=10), respectively. Because steroid hormones have a gut passage time of approximately 24 hours in geladas (Beehner and McCann, 2008), all fecal estradiol hormone values are assigned a date one day prior to fecal collection for all analyses.

Sampling schedules

Demographic, behavioral, and sexual swelling data were collected opportunistically (everyday possible) for all females throughout the project. From Apr 2007-Dec 2008, females from two units were targeted for fecal sample collection once per month (10-16 females at a time). During 2009 and 2010, females were selected (based on reproductive stage, see below) for high, medium, and low sampling coverage (see Table 2.1 for a summary of fecal sampling). With a few exceptions, all fecal samples were accompanied by at least one focal sample and detailed description of reproductive condition. For examining the characteristics of ovarian cycles, 10 parous females (who were presumed to be cycling) were targeted for high coverage

and were sampled every day possible (averaging 1 sample every 2 days). For tracking gestation, 5 females (who were presumed to be pregnant) were targeted for medium coverage, sampling twice per week (averaging 1 sample every 4 days). For tracking lactation, all samples ($N = 59$) were drawn from the low coverage group (averaging 1 sample every 30 days) and supplemented with samples from other lactating females that were not members of the closely followed units. One important note, *in no case* did we use samples collected during the 3 months following takeovers for any of our analyses. These periods of potential instability will be addressed in later chapters.

Maturation Milestones

Age at maturation was calculated as the age at which a female first exhibits a sexual swelling. Only a subset of females with known birth dates were used ($N = 11$). Similarly, age at first birth was calculated for females with known birth dates and known dates of parturition. As of the start of this analysis (June 2012), only two females born during our study period had given birth. Therefore, we bolstered this sample size with additional females estimated to be <6 months old at the start of the long-term project. This provided an additional two females to the sample size ($N = 4$).

Reproductive parameters: length of time

Interbirth interval. The interbirth interval (IBI) for a female is the time in days from the birth of one infant to the birth of her next infant. Intervals were excluded from analyses if (1) the first infant in the sequence was not successfully-weaned (but died prior to this point), or (2) the second pregnancy in the sequence was not carried to term (i.e., miscarriage). Furthermore, IBIs were calculated only when *both birthdates* in the sequence were known ($N = 51$) and not estimated. In cases where one female had more than one IBI, we calculated a mean IBI per

female prior to calculating a mean for the population. In this way, we ensured that each female contributed equally to the population mean.

Ovarian cycle length. We used ovulatory estrogen peaks to determine the length of the gelada ovarian cycle (as in Yan & Jiang, 2006; Higham et al., 2008; Lu, Borries, Czekala, & Beehner, 2010). Based on our high-sampled females, fecal estradiol (fE) levels for the periovulatory period (POP, defined below) had a mean of 21.45 ± 4.95 ng/g ($N = 7$). This was well above non-periovulatory levels (mean fE = 4.98 ± 0.69 ng/g; $N = 7$) (Roberts et al., 2012). The date of ovulatory peak was assigned as the day of highest fE value for that female that fell more than 1 SD above the mean periovulatory value. Cycle length was defined as the number of days between two successive fE peaks (see Figure 2.3 for visual representation).

Determining the ovarian cycle length using hormonal sampling for a wild primate is much more difficult than might be expected. For example, of the 10 parous females that we targeted on a near-daily basis for measuring cycle length, 2 were pregnant before sampling began, 2 had no discernible fE peaks during sampling (possibly due to infertility), and 3 became pregnant after the first ovulatory peak (see definition of conception below). Therefore, of these 10 females, only 3 exhibited a non-conceptive ovulatory peak; and of these 3, only one exhibited 2 peaks successively (reducing the sample size from 10 down to 1). Fortunately, an additional 2 females from the opportunistic sampling group were found to have consecutive peaks with non-periovulatory fE levels between peaks. Therefore, our sample for calculating gelada cycle length was 3 females.

In addition to the overall length of the ovarian cycle, we also broke this down into its components and calculated the lengths of the follicular phase and the luteal phase. The follicular phase is marked by the recruitment and development of oocytes and the resultant increase in

estrogens released from the developing follicles (Johnson, 2007). The follicular phase ends with the ovulatory peak in estrogens which, in turn, results in a peak in luteinizing hormone (LH) from the anterior pituitary. The high levels of LH cause follicular rupture and the release of the ovum (i.e., ovulation) within 1-2 days of the fE peak. The luteal phase follows ovulation and is marked by low fE levels when the remaining follicular structure becomes the corpus luteum and mainly produces progesterone (Johnson, 2007). Here, we assumed that the first rise in fE after the postovulatory dip from the previous cycle (more than 2 SD above the preceding 3 values) indicates the start of follicular development for the current cycle (as per Lu et al., 2010, see Figure 2.3). Therefore, the length of the follicular phase was calculated as the number of days from the first day of the fE rise through the day of the ovulatory peak. The length of the luteal phase was calculated as the number of days between the ovulatory peak to the day before the next fE rise (Figure 2.3).

Gestation length. Conception was defined as the day of the ovulatory fE peak (as defined above) when it was followed by increasing levels of fE above follicular levels (see below). Gestation length was calculated as the number of days between conception and the known date of parturition. Our sample size for this includes 6 females (5 with low coverage for hormone sampling and 1 with high coverage).

Postpartum amenorrhea. Postpartum amenorrhea was calculated as the number of days from parturition to the first day a female was recorded with a sexual swelling ($N = 31$). This was calculated only following pregnancies with known infant birthdates. Cases were excluded if: (1) infant death occurred before weaning, (2) no swelling was observed between births, or (3) a takeover occurred during lactation; (Because female geladas are thought to exhibit “deceptive” sexual swellings, see Chapter Five, we did not want these false swellings to bias our sample of

true postpartum amenorrhea). When females had more than one period of amenorrhea, we calculated a mean per female prior to calculating a mean for the population.

Time to conception. We calculated the time to conception as the number of days from a female's first postpartum sexual swelling until her estimated date of conception ($N = 35$). Because we did not have high-coverage hormone sampling from all females to ascertain the exact onset of gestation, we assigned an estimated date of conception based on the average length of gestation for this population (see results). Given how invariable gestation length is within a species (Kiltie, 1982), we feel that this estimate is justified. We excluded any cases of known pregnancy loss as the date of conception could not be estimated with precision. Likewise, any cases of infant death were excluded because in some species the time to conception is greater following infant loss than following normal weaning (e.g., anubis baboons, Higham et al., 2009). In addition, any females that were observed less than twice per month (occasionally, units "disappear" to the lowlands during the dry season) were not included due to the higher risk that the first postpartum swelling was missed. When females had more than one value, we calculated a mean per female prior to calculating a mean for the population.

Reproductive Parameters: Hormones and Behavior

Cycles. Average fE values were calculated for the ovulatory peak, luteal, and follicular phases according to the definitions above (see section on ovulatory cycle length). Peak fE ($N = 7$) was determined using 3 conceptive and 4 non-conceptive (2 from the same female) cycles. The luteal phase fE was, by necessity, calculated only for the non-conceptive cycles ($N=3$). The follicular phase fE value ($N=4$) was calculated using all non-conceptive cycles and one conceptive cycle for which sampling was sufficient to ensure that we could identify the

beginning of the follicular phase (i.e., we had the end of the last ovulatory rise and the post-ovulatory dip).

For examining variation in sexual swellings and behavior across the cycle, we divided cycles into three distinct periods. Note that in contrast to the division detailed above (where the ovarian cycle is divided into the follicular phase and the luteal phase), this division separates the ovarian cycle into three different periods based on what we expect in terms of behavior. The periovulatory period (POP) is defined as the 5-day period starting 3 days before ovulation and ending one day after ovulation (as per Deschner, Heistermann, Hodges, & Boesch, 2003; Higham et al., 2008). The POP is the assumed period of fertility based on the timeline of potential conception in humans relative to the life spans of released eggs (which are generally fertile for a period of no more than 24 hrs; France, 1981) and ejaculated sperm (which are generally potent for 3 days after insemination; Wilcox, Weinberg, & Baird, 1995). The period leading up to the POP is called the “early follicular” (EF) period and included all days between an identified follicular rise in fE (see section on ovulatory cycle length above) and the POP. The “late luteal” (LL) period included all days between the POP and the follicular rise in fE. Data on sexual swellings and sexual behaviors are described for the EF ($N=4$), POP ($N=6$), and LL ($N=3$) periods.

Pregnancy. Although we calculated gestation length based on females with frequent hormone sampling at the time of conception (see above), we broadened our data set in this analysis to include all pregnant females. For any females without adequate hormone sampling at the time of conception, we estimated conception based on the mean gestation length ($N = 53$). All hormone samples, sexual swellings, and behavioral samples between the estimated conceptions and known birth dates were included in the pregnancy composite profiles presented

here. Profiles are presented as weeks relative to birth. In cases where females had more than one sample/week, a mean value was taken for each measure and used to avoid any bias from high-coverage females. To examine variation in fE, sexual swellings, and sexual behavior across pregnancy, we separated pregnancy into three 2-month trimesters for analysis. Here, sampling was balanced by calculating a mean for each measure in each trimester for each female.

Lactation. All hormone samples, swellings, and sexual behavior during a female's postpartum amenorrhea were included in the lactation composite profiles. Profiles are presented in postpartum months. In cases where females had more than one sample/month, a mean value was taken for each measure and used to avoid any bias from high-coverage females. To examine variation in fE, sexual swellings, and sexual behavior across lactation, we separated lactation into the first 12 months of lactation and all time over 12 months but before weaning (there was high variability in length of postpartum amenorrhea). Here, sampling was balanced by calculating a mean for each measure in each period.

Results

Maturation milestones and reproductive parameters

A summary of maturational milestones and reproductive parameters is given in Table 2.3.

Fecal estrogens

Descriptive statistics for fE values across cycling, gestation, and lactation are listed in Table 2.4, and a visual representation of these values across the cycle (broken down by the behavioral categories: EF, POP, and LL), pregnancy, and lactation, are illustrated in the top panel of Figures 2.4, 2.5, and 2.6 respectively. Mean fE increases beyond 2 SD above mean follicular levels in week 3 of gestation, averaging 17.85 ng/g (SD = 39.36), and above ovulatory peak levels in week 8 of gestation, averaging 72.22 ng/g (SD = 55.40). Mean fE values across

lactation are 4.28 ng/g (SD = 8.65). After removing values from the first 2 days postpartum, this drops to 2.26 ng/g (SD = 1.52). To facilitate comparisons across the reproductive stages, Figure 2.7 shows broad patterns across the partitions of all stages.

Sexual swellings

Descriptive statistics for sexual swelling scores across the reproductive stages are listed in Table 2.5 and a visual representation of some of these values across the cycle (broken down by the behavioral categories: EF, POP, and LL), pregnancy, and lactation, are illustrated in of Figures 2.4, 2.5, and 2.6, respectively. Remarkably, across all reproductive stages, there was little variation in chest color (Figure 2.7).

Cycling females had chest vesicles throughout the cycles, yet there was very little variation in the vesicle organization, with 91% of samples exhibiting the “K” organization (Figure 2.2). In contrast, mean turgidity scores suggested more of a pattern with highest turgidity emerging in the EF and POP periods of the cycles compared to all other reproductive stages.

Surprisingly, pregnancy was also marked by sexual swellings for nearly all pregnant females. Only 1 out of the 53 females was never found to be swelling during her pregnancy. The “K” organization was also the most common organization for the first 2 trimesters of pregnancy, although organization in general was much more varied across pregnancy than across cycling. Average vesicle turgidity was comparable to the LL cycling period for the 1st and 2nd trimesters. Vesicle organization was much more variable in the third trimester. While the most common organization was “A” (28.5%), a completely flat chest without vesicles, overall turgidity scores only dropped to 1.2 for the third trimester (i.e., 71.5% of samples included some vesicle swelling during this trimester).

Lactating females exhibited dramatic differences from cycling and pregnant females with respect to sexual swellings. The vast majority of females had no vesicles during lactation. Three females were found swelling once in their lactational period, then returned to a flat chest. In all cases the turgidity scores were 1; the vesicles were wrinkled and empty.

The paracollousal skin was much less variable than the chest patch. Nevertheless, several patterns are noteworthy. First, our limited samples of scaled paracollousal color indicate that the paracollousal skin peaks (in redness) in late pregnancy and drops at the beginning of lactation. Second, perineal vesicles are virtually limited to cycling and early pregnancy. Third, menstruation was only observed for 1 cycling female (occurring from days 17-23 post-ovulation), despite all cycling females being observed on a near-daily basis.

Overall, there was very little variation in the vesicle organization and paracollousal skin state across the reproductive stages and the sample sizes were very low for the scaled paracollousal color. Therefore, further discussion of sexual swellings centers on only chest color, vesicle turgidity, and the presence of perineal vesicles.

Sexual behavior

Sexual behavior was observed across all reproductive stages (Figure 2.7, panel B). For cycling females, as expected, the majority of sexual behavior was observed before and around the POP (Figure 2.4, panel B). Presenting to the male was the only behavior seen more than 2 days into the LL period. One female was never observed producing any sexual behavior. The remaining 5 females were all observed producing pre-copulatory calls and copulating, only 2 presented during this time.

Sexual behavior was also observed across all stages of pregnancy (Figure 2.5, panel B). A total of 7 females displayed pre-copulatory calls (all during the first trimester). Eleven females

presented during pregnancy (4 in the 1st trimester, 3 in the 2nd trimester, and 4 in the 3rd trimester. Eight females copulated during pregnancy (3 within the first 3 weeks of gestation, the other 5 copulated during the 1st and 2nd trimester).

In sharp contrast with cycling and pregnancy females, lactating females exhibited very little sexual behavior. Two females produced pre-copulatory calls (postpartum months 16, 18), 3 females presented to males (months 2, 11, 12, 16 and 18); 1 female copulated (month 16). Accounting for the vast majority of sexual behavior during lactation, one female produced 8 out of the 11 total behaviors recorded during across lactation and she is the only female that copulated before her first postpartum swelling.

Discussion

This is the first study to use steroid hormone analysis to define reproductive parameters in wild female geladas. We successfully utilized fE to identify ovulatory peaks as well as pregnancy (and pregnancy failure, as Chapter Four will detail).

In comparison with the life history estimates from Dunbar (1980) on the same population of geladas, several parameter values were confirmed (see Table 2.6 for comparisons). Cycle length, gestation, and IBI were very similar between this and Dunbar's study. Not surprisingly, the parameters that rely on accurate age estimates in Dunbar's study were less similar to our values (from female with known ages). For example, our mean age at first birth was 5.2 years, almost a year older than Dunbar's estimate. Furthermore, this age at first birth probably represents an *underestimate*, considering that our current dataset is necessarily biased towards females that give birth early. Once the other 7 females that were < 6 months at the beginning of the project and more females that were born during the project give birth, our age at first birth in geladas will be more accurate. Additionally, our length of postpartum amenorrhea (1.3 years)

was shorter than Dunbar's estimate (1.64 years). Third, our time to conception (241.86 days) was almost triple Dunbar's estimate (87.5 days).

Compared to baboons, geladas have several life history parameters consistent with *Papio* spp. ("baboons"), as well as several interesting differences (see Tables 2.7 and 2.8 for comparative data). The gelada cycle length and gestation length fall completely within the available ranges for baboons. The mean gelada postpartum amenorrhea falls within the ranges of the baboons, but is slightly higher than the means for those species. However, the average gelada ages at maturation and first birth were lower than those reported for baboons. The mean age at maturation for gelada females is 4.2 years. This is most similar to hamadryas baboons that mature around 4.3 years (Sigg et al., 1982), but lower than anubis and yellow baboons (5.0 and 4.9 years, respectively; Packer, 1979; Rhine, Norton, & Wasser, 2000). It is noteworthy that only one of our females fell outside the entire baboon range (4.0-5.6), with an age at maturation at 3.5 years. Our estimated age at first birth for geladas is 5.2 years, compared to 6.1 years in hamadryas (Sigg et al., 1982) and 6.5 in yellow baboons (Rhine et al., 2000). Again, this may be due to the small sample size and a bias in our current data set.

We found the average gelada IBI to be almost 915 days, which is longer than IBIs reported for baboons (means range from 519-852 days; Table 2.8). Considering the similar lengths in gestation and postpartum amenorrhea, this difference may be due to geladas' longer time to conception. We found an average of 242 days to conception in our population while baboon averages range from 122-170 days (Table 2.8). Together with the high level of variability in the time to conception, we found that the minimum gelada IBI (411 days) is not outside the overall range found for hamadryas baboons (350-1098 days; Sigg et al., 1982; Swedell, 2005),

but the maximum gelada IBI (1546 days) is over a year longer than has been reported for any baboon species (Table 2.8).

Our data on cycling parameters were partially consistent with those from the captive study by McCann (1995). Our overall cycle length was almost 35 days while McCann found it to be 40. However, we found shorter lengths for both the follicular and luteal phases compared to McCann as well. Our follicular and luteal phases were 18.0 and 11.3 days, respectively, while McCann found 20.0 and 18.0 days lengths for these phases. Because our sample size is small ($N = 3$), sampling from more females will enable us to determine whether this is a sampling effect or if wild female geladas have shorter cycles than those in captivity. The results on turgidity and chest color are very similar between these two studies. Females are generally swollen throughout their cycle, but are only at peak swelling for the pre ovulatory and ovulatory periods. However, McCann found that peak turgidity only lasted 7 days whereas we found peak swelling to last more than 14 days. Further, while McCann found that females copulate across the entire ovulatory cycle, this was not seen in our data. Females only mated between 17 days prior to ovulation and 3 days following ovulation. Again, this may be an artifact of our low sample size. Another surprising finding from this study was the rarity of observed menstruation. Only one female was found menstruating amongst the 3 non-conceptive cycles. Menstrual discharge is relatively low in geladas compared to baboon species (Matthews, 1956), therefore it is likely that we missed the presence of menstrual blood in the other cases.

The largest caveat with our cycle analysis is that we lack direct evidence of ovulation. We assume that the peak in fE was sufficient to indicate the occurrence of ovulation. To be certain, we would need evidence of the formation of the corpus luteum, the structure that remains after the ovum is released from the follicle (i.e., ovulation). The corpus luteum is responsible for

increased levels of progesterone which allow for implantation and the beginning of pregnancy. Therefore if we had fecal levels of progesterone or its metabolites, we could say with certainty that ovulation had occurred. The critical implication of this caveat is that we cannot say for certain that the non-conceptive cycles were indeed ovulatory. Although we have had some difficulty finding a sensitive antibody for progesterone, assaying all samples for progesterone remains a high priority for future work.

Our pregnancy data indicate that fE levels rise above follicular levels in week 3 of pregnancy, but only rise above periovulatory levels in week 8. This has implications for identifying pregnancy in captive breeding programs and monitoring wild populations. Despite high variability, fE values across pregnancy steadily increase, similar to that shown for yellow baboons (Beehner, Nguyen, Wango, Alberts, & Altmann, 2006). While multiple samples across weeks 3-7 within an individual would show a rise above follicular fE levels and indicate pregnancy, a single fE sample would not be able to distinguish pregnancy from ovulation until week 8 or beyond.

Another, rather surprising, finding that is problematic for identifying pregnancies is that both the sexual swellings and the sexual behavior across pregnancy were similar to that observed for cycling females. Sexual swellings throughout pregnancy were the norm rather than the exception. Perhaps the best data on the sexual swellings that may indicate broad reproductive stage was vesicle turgidity (Figure 2.7). The turgidity of vesicles for cycling and pregnant females decreased both within and across stages. Again, as with fE, long-term tracking of turgidity within individuals would be the only way to identify changes between stages, and a single swelling sample could not differentiate pregnancy from cycling. Similarly, the absence of vesicles is not clearly indicative of reproductive stage as females were found in all stages without

vesicles (i.e., not swelling). There were also low levels of sexual behavior throughout pregnancy. Although it is possible that sexual behavior in the first 3 weeks of the first trimester could be related to the female's conceptive ovulation (our estimated conception dates come from the population mean and may be earlier than actual conception), both copulations and presents are seen into the 2nd and 3rd trimesters, respectively.

In stark contrast to our pregnancy results, the lactating female results were exactly as expected. We found very low levels of fE, and both sexual swellings and behavior were, in effect, absent during lactation. This is expected because lactation is generally the most expensive stage of reproduction amongst mammals. During this time, females are unlikely to put energy into producing sexual swellings or behavior while investing in milk production.

The presence of sexual swellings and behavior throughout pregnancy signify that, in geladas, these are not honest signals of fertility (Nunn, van Schaik, & Zinner, 2001). In other species, such 'deceptive' indicators of fertility (i.e., in the absence of ovulation) are associated with post-conceptive mating that serves to confuse paternity and decrease the chances of infanticide (e.g., Hrdy, 1979). However, signals must be reliable and honest the majority of the time if they are to continue functioning. Further, when deception does occur, it should be at low rates to maintain the signal's original function (Stuart-Fox, 2005). For geladas in stable single-male groups or in stable groups with follower males, confusing paternity would seem unnecessary because no infanticides have been reported except following takeovers (e.g., Beehner & Bergman, 2008). Therefore, we would expect deceptive sexual swellings and behavior to occur only when social factors increase the chances of infanticide (i.e., after a male takeover). Here, the observed deceptive swellings and sexual behavior during pregnancy occurred in the absence of takeovers. Nevertheless, these post-conception copulations indicate

that the males are unable to identify conceptive versus non-conceptive females, and that deceptive sexual swellings and behavior may be a counterstrategy to infanticide following takeovers (see Chapter Five for further discussion).

In conclusion, this is the first study to explore female reproductive parameters using non-invasive physiological monitoring techniques in wild geladas. This study provides baseline data for exploring the evolution of reproductive and mating systems across species as well as identifying reproductive strategies within geladas. Additionally, considering the increasing threat to gelada conservation, a baseline of reproductive parameters is perfectly timed to inform conservation management decisions and increase their efficacy.

Table 2.1. Summary of fecal hormone sampling.

		UMGRP (Apr 2007-Dec 2008)		This study (Jan 2009-Dec 2010)	
Target sampling		Females	Average samples per female	Females	Average samples per female
Low-coverage	1 sample/month	15	21.3	52	13.4
Mid-coverage	2 sample/week	--	--	5	16.6
High-coverage	1 sample/day	--	--	10	33.1

Table 2.2. Ethogram of sexual behavior.

Proceptive behaviors	Definition
Pre-copulatory call	A series of short, uttered calls separated by inhalations. Pre-copulatory calls are characteristic of estrous and function to convey information about female status to the male (Moos-Heilen and Sossinka, 1990).
Present	The female turns her genitalia, chest patch, or abdomen directly to the male while within 1m. This can also be accompanied by using her hands to move the male's head toward the presented portion of her body.
Receptive behavior	Definition
Copulation	Intromission by the male. The female is standing on all four limbs, the male will stand on their two rear legs, hold the female's hips with his hands, mount, and thrust. Copulations are often accompanied by copulatory calls by both the male and female.

Table 2.3. Summary of female gelada maturational milestones and reproductive parameters.

Maturational milestones	Mean \pm SD	Median	Range	N
Age at maturation (in years)	4.19 \pm 0.32	4.16	3.48-4.64	11
Age at first birth (in years)	5.20 \pm 0.36	5.16	4.82-5.68	4
Reproductive parameters	Mean \pm SD	Median	Range	N
Interbirth interval (days)	900.1 \pm 232.0	903.0	411.0-1546.0	45
Cycle length (days)	34.3 \pm 6.7	31.0	30.0-42.0	3
Follicular phase length (days)	18.0 \pm 5.3	16.0	14.0-24.0	3
Luteal phase length (days)	11.3 \pm 4.0	12.0	7.0-15.0	3
Gestation length (days)	182.7 \pm 6.7	184.5	173.0-189.0	6
Postpartum amenorrhea (days)	481.7 \pm 147.2	507.5	254-736.0	22
Time to conception (days)	241.9 \pm 185.4	181.0	4.0-638.5	35

Table 2.4. fE across the female reproductive stages.

Reproductive stage	Mean fE (ng/g) \pm SD	Median	Range	N
Cycles				
Follicular phase	12.30 \pm 1.45	12.10	10.96-13.84	3
Peak	36.90 \pm 7.41	34.36	30.47-52.92	7
Luteal phase	5.49 \pm 1.46	5.28	4.14-7.03	3
Gestation				
1 st trimester	38.06 \pm 30.00	29.43	4.09-128.03	41
2 nd trimester	96.42 \pm 37.15	90.66	37.83-214.50	42
3 rd trimester	136.11 \pm 60.74	120.70	50.89-320.44	44
Lactation				
1 st 12 months	3.73 \pm 7.30	2.03	0.82-41.59	57
> 12 months	2.04 \pm 1.98	2.10	1.12-10.90	23

Table 2.5. Sexual swelling scores across the female reproductive stages.

Reproductive stage	Chest color (1-5 scale) Mean \pm SD	Organization (most common) Score (% of total samples)	Chest vesicle turgidity (0-3 scale) Mean \pm SD	Paracollousal skin state % mottled out of total samples	Paracollousal skin color (1-5 scale) Mean \pm SD	Perineal vesicles % present out of total samples
Cycles						
EF	3.2 \pm 0.5	K (77.8%)	2.4 \pm 0.6	100.0%	2.2 \pm 0.3	15.8%
POP	3.0 \pm 0.9	K (95.5%)	2.4 \pm 0.8	86.4%	2.5 \pm 0.04	63.6%
LL	2.3 \pm 0.2	K (100.0%)	1.6 \pm 0.3	100.0%	2.5 \pm 0.7	51.9%
Gestation						
1 st Trimester	2.8 \pm 0.6	K (23.0%)	1.6 \pm 0.7	84.6%	2.0	12.8%
2 nd Trimester	2.8 \pm 0.5	K (17.0%)	1.5 \pm 0.7	67.6%	3.2 \pm 0.3	0.0%
3 rd Trimester	2.8 \pm 0.7	A (28.5%)	1.2 \pm 0.8	53.5%	3.4 \pm 0.6	0.6%
Lactation						
1 st 12 months	2.3 \pm 0.1	A (99.7%)	0.0 \pm 0.0	91.8%	2.0 \pm 0.3	0.0%
> 12 months	2.2 \pm 0.1	A (99.3%)	0.0 \pm 0.0	92.2%	1.8 \pm 0.4	0.6%

Table 2.6. Life history parameters from this study and Dunbar (1980). To be consistent, values from this study are shown in the units of time presented by Dunbar.

Life history parameter	This study Mean \pm SD	Dunbar (1980)
Age at first birth (years)	5.2 \pm 0.4	4-4.5
Interbirth interval (years)	2.5 \pm 0.6	2.4
Cycle length (days)	34.3 \pm 6.7	35.0
Gestation (months)	6.0 \pm 0.2	6.0
Postpartum amenorrhea (years)	1.3 \pm 0.4	1.6
Time to conception (days)	241.9 \pm 185.4	87.5 (converted from number of cycles)

Table 2.7. Summary of gelada and baboon maturational milestones.

Age at maturation					
Species	Mean	Range	N	Age determination	Source
Geladas	4.2	3.5-4.6	11	known birth dates	This study
Baboons:					
Hamadryas	4.3	4.0-5.0	13	phys chars/body size	Sigg et al., 1982
Anubis	5.0	4.5-5.6		unspecified	Packer, 1979
Yellow	4.9	4.7-5.2 ¹	29	known birth dates	Rhine et al., 2000
Age at first birth					
Geladas	5.2	4.8-5.7	4	known/estimated birth dates	This study
Baboons:					
Hamadryas	6.1	5.5-7.0 ¹	8	phys chars/body size	Sigg et al., 1982
Anubis	-	-	-	-	-
Yellow	6.5	6.1-6.9	29	known birth dates	Rhine et al., 2000

¹ 95% confidence interval, not range

Table 2.8. Summary of gelada and baboon reproductive parameters. To be consistent, values are shown rounded to the closest day.

Interbirth interval				
Species	Mean	Range	N	Source
Geladas	915	411-1546	51	This study
Baboons:				
Hamadryas	732	549-1098	16	Sigg et al., 1982
	519	350-656	4	Swedell, 2005
Anubis	760	NR	23	Smuts & Nicholson, 1989
Yellow	852	439-1095	13	Bentley-Condit & Smith, 1997
	641	NR	9	Altmann et al., 1977
Cycle length				
Geladas	34	30-42	3	This study
Baboons:				
Hamadryas	40	23-60	39	Swedell, 2005
				Smuts & Nicholson, 1989
Anubis	42	NR	33	
Yellow	39	14-68	12	Bentley-Condit & Smith, 1997
	33	26-52	26	Hausfater, 1975
Gestation length				
Geladas	183	173-189	6	This study
Baboons:				
Hamadryas	-	-	-	-
Anubis	180	NR	13	Smuts & Nicholson, 1989
Yellow	182	NR	28	Bentley-Condit & Smith, 1997
	175	NR	23	Altmann et al., 1977
Postpartum amenorrhea				
Geladas	482	254-736		This study
Baboons:				
Hamadryas	427	305-488	13	Sigg et al., 1982
	268	265-268	2	Swedell, 2005
Anubis	407	NR	61	Smuts & Nicholson, 1989
Yellow	445	244-702	22	Bentley-Condit & Smith, 1997
	375	183-488	20	Altmann et al., 1977
Time to conception				
Geladas	242	4-639	35	This study
Baboons:				
Hamadryas	-	-	-	-
Anubis	170	NR	39	Smuts & Nicholson, 1989
Yellow	150	16-570	24	Bentley-Condit & Smith, 1997
	122	30-259	27	Altmann et al., 1977

Figure 2.1

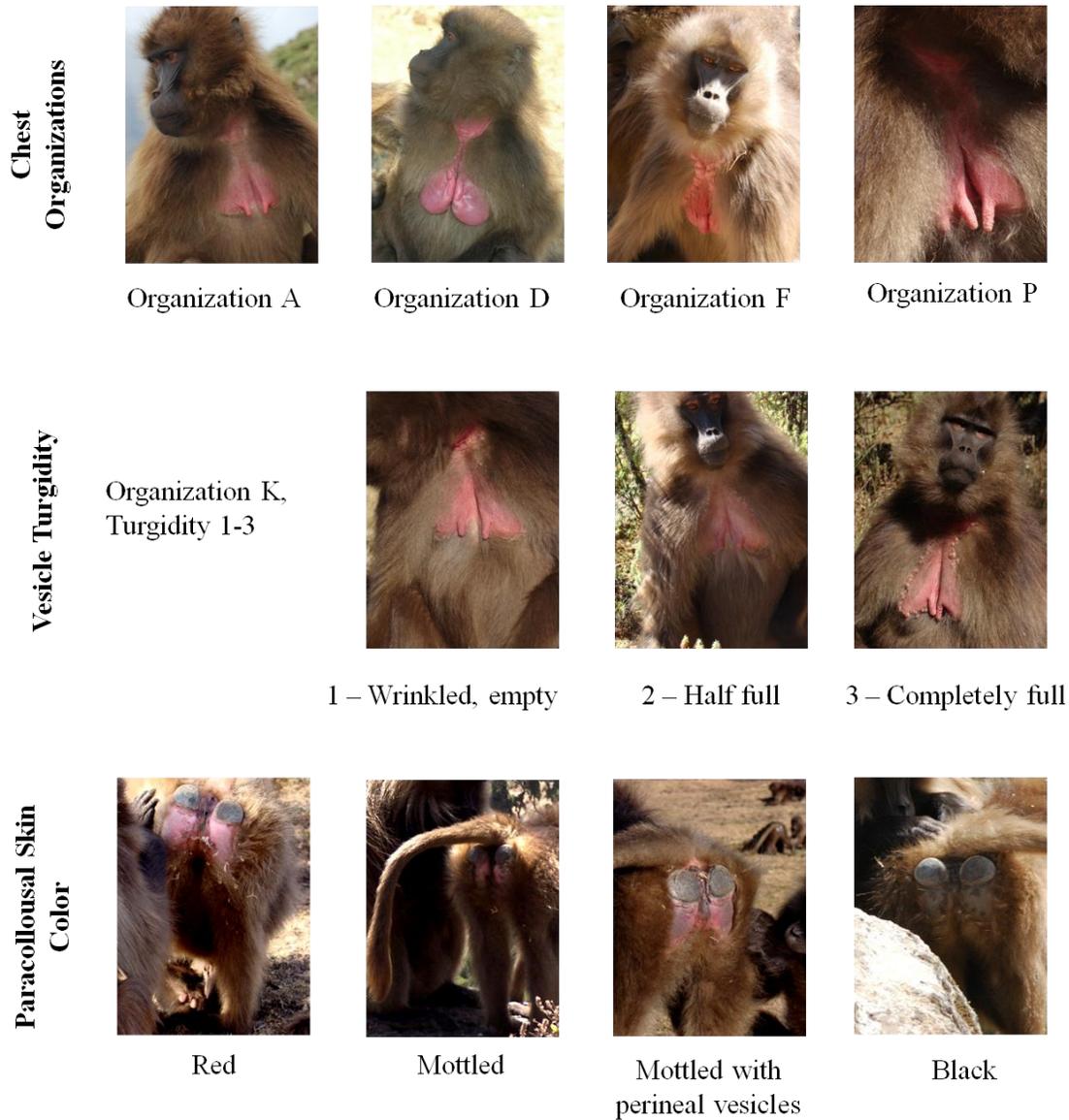


Figure 2.1. Representative images of gelada sexual swellings. First row: Selected organizations of chest swellings. Second row: Variation in vesicle turgidity in one of the most common sexual swelling organizations, K. See “Organization A” in the first row for an example of “0” turgidity. Third row: variation in paracollousal skin color. See Figure 2.2 for complete catalog of chest scores and color scale.

Figure 2.2. Chest sexual swelling score sheet. Each chest sample included three scores: (1) color on a scale of 1-5, (2) organization from A-P, and (3) vesicle turgidity on a scale from 0-3. In addition, the paracollousal skin scores included the presence/absences of perineal vesicles and broad color (see Figure 2.1). Beginning in January 2010, paracollousal color was also scored for specific color using the color scale pictured here.

Figure 2.2

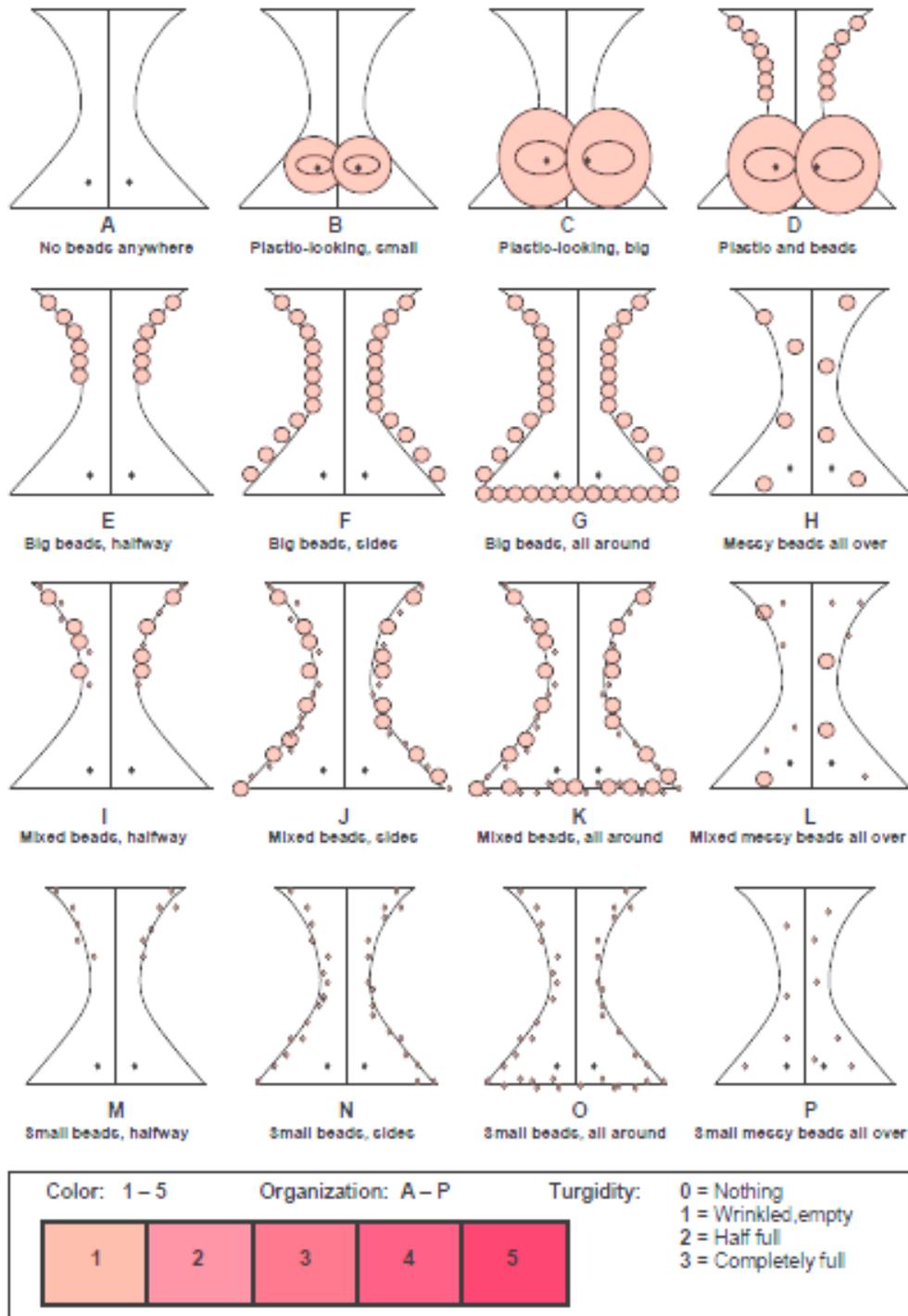


Figure 2.3

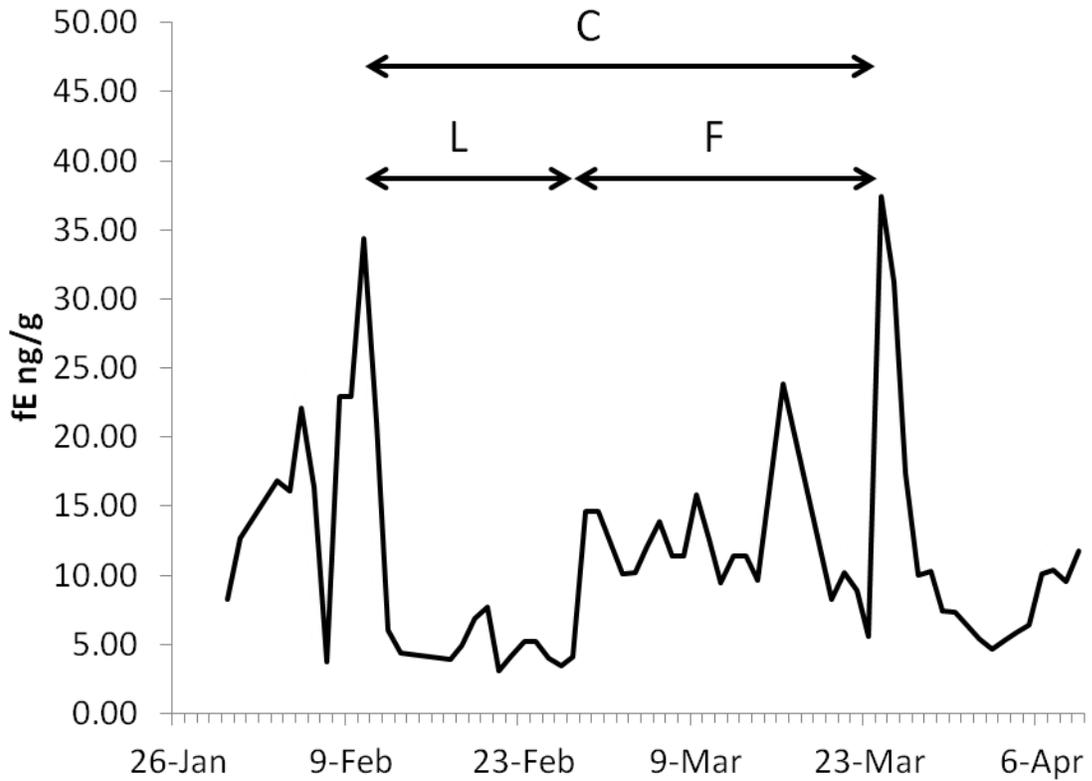


Figure 2.3. Fecal estrogen profile of one female to outline cycle parameters. Cycle length (C), luteal phase length (L) and follicular phase length (F) were determined using fE values (see text for description).

Figure 2.4. Characteristics of the ovarian cycle. Each profile of the cycle is based on a combination of longitudinal and cross sectional data centered on ovulation. Panel A shows mean $fE \pm SEM$ for all females ($N = 6$). Following the ovulatory peak the lines are separated such that non-conceptive cycles ($N = 3$) continue to represent a normal cycle (in black) and the early pregnancy is shown for conceptive cycles ($N = 3$; in gray). For the remaining characteristics, all cycling females were included in the periovulatory period (POP; $N = 6$) while only females with identified follicular and luteal phases were included in the early follicular (EF) and late luteal (LL) periods ($N = 3$). The black bars in panel B indicate the presence of each characteristic scored with presence/absence or 1-0 sampling. In cases of females not observed on a single day in between two days of presence or observation of the measure, presence or observation was assumed for that middle day. Panels C and D show the mean chest color and vesicle turgidity scores $\pm SEM$ across the gelada cycle.

Figure 2.4

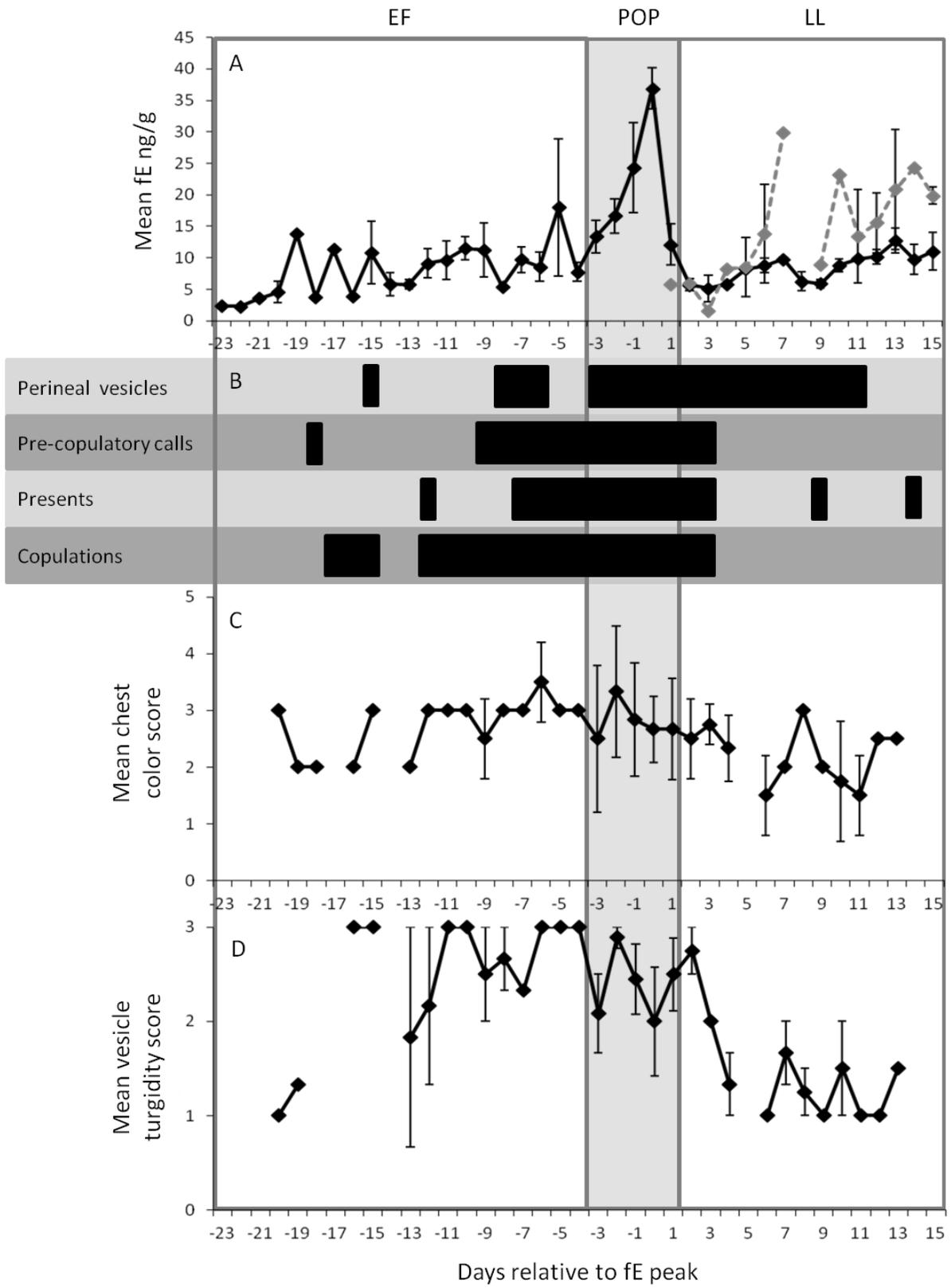


Figure 2.5. Characteristics of pregnancy. Each profile of pregnancy is based on a combination of longitudinal and cross sectional data ($N = 53$). Panels A, C, and D show the mean \pm SEM for fE, chest color, and vesicle turgidity scores, respectively. The black bars in panel B indicate the presence of each characteristic scored with presence/absence or 1-0 sampling on a weekly basis.

Figure 2.5

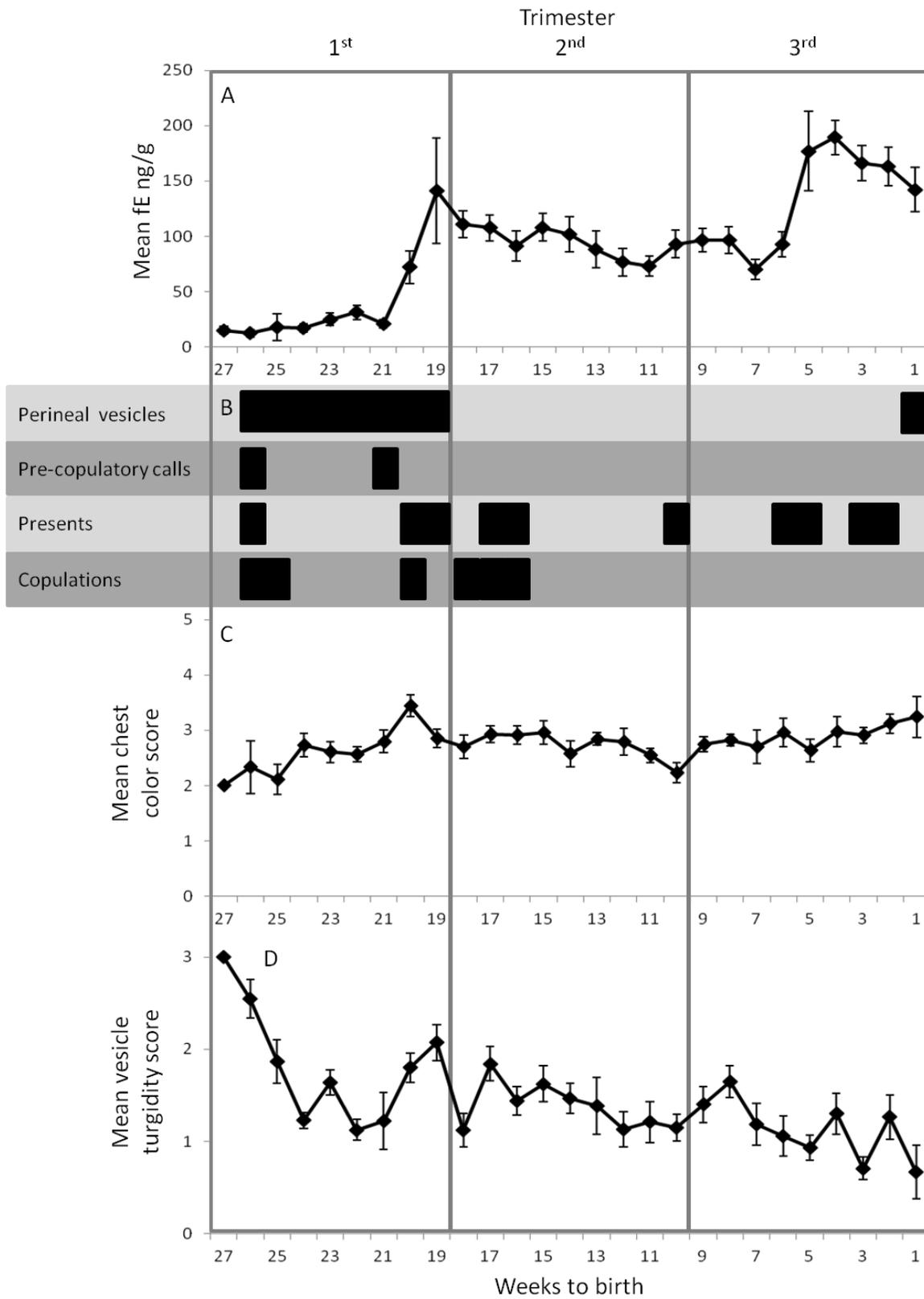


Figure 2.6. Characteristics of lactation. Each profile of lactation is based on a combination of longitudinal and cross sectional data ($N = 50$). Panels A, C, and D show the mean \pm SEM for fE, chest color, and vesicle turgidity scores, respectively. The black bars in panel B indicate the presence of each characteristic scored with presence/absence or 1-0 sampling on a monthly basis.

Figure 2.6

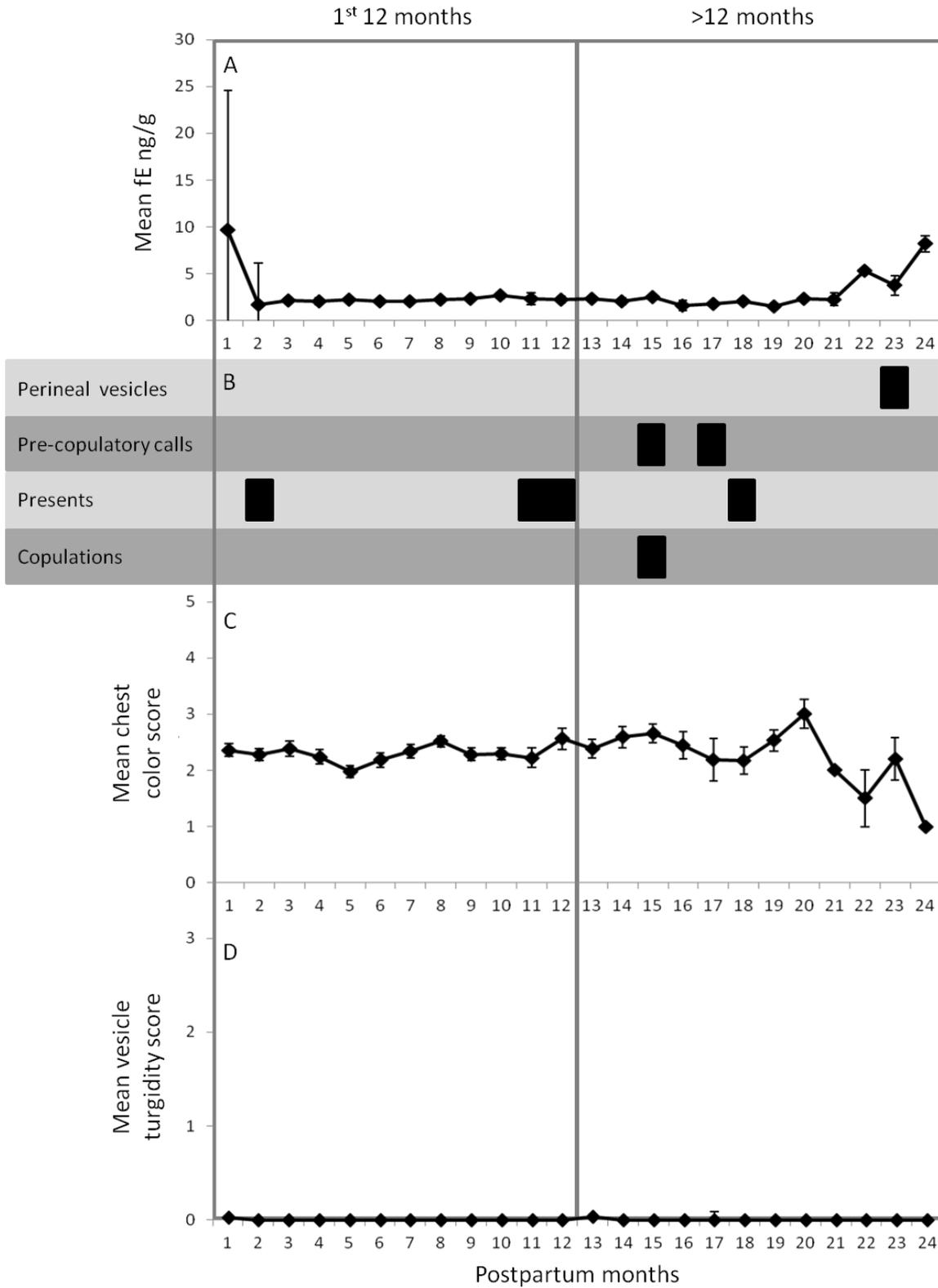
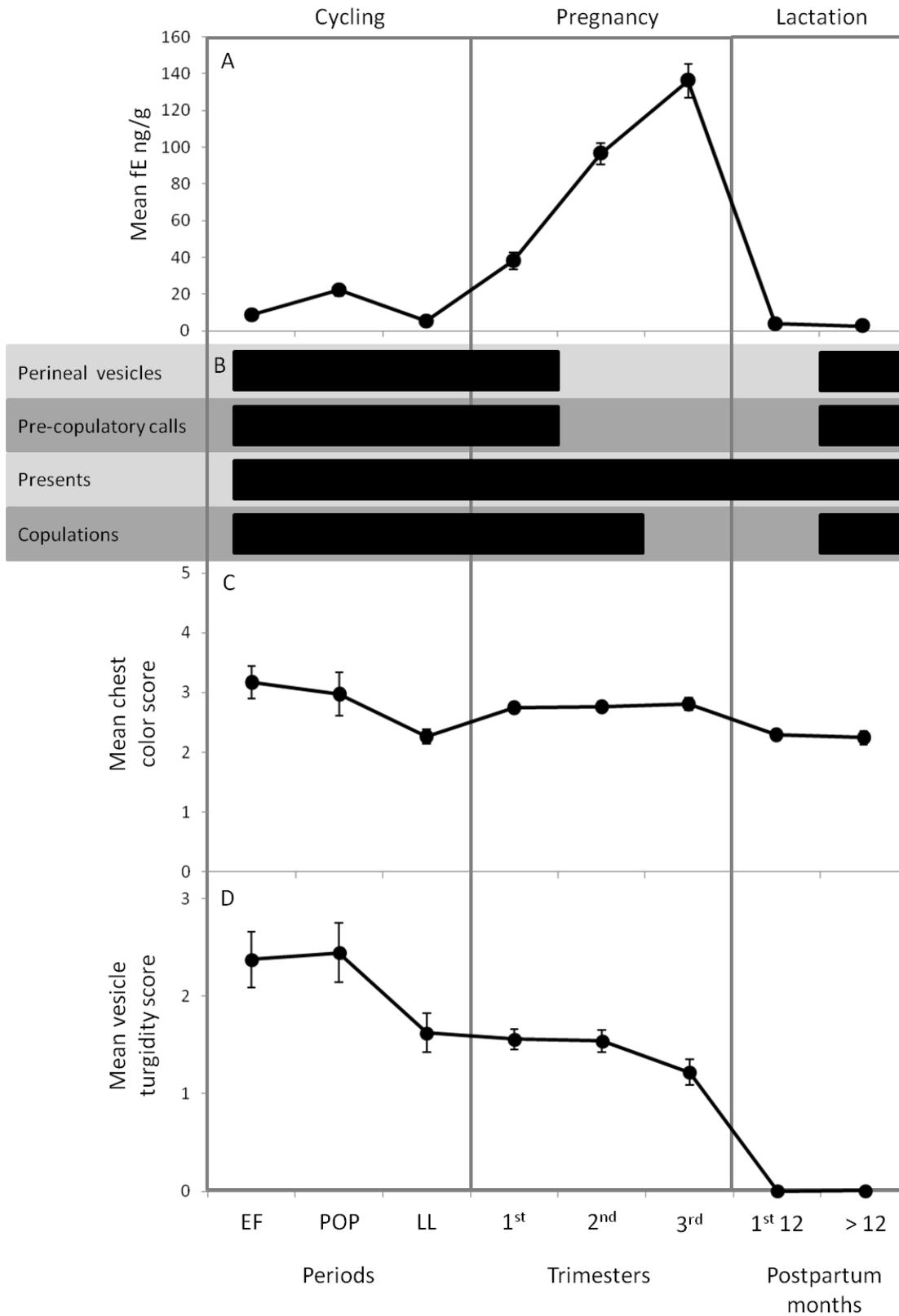


Figure 2.7. Characteristics across the reproductive stages. Each profile across the reproductive stages uses a combination of longitudinal and cross sectional data. Panels A, C, and D show the mean \pm SEM for fE, chest color, and vesicle turgidity scores, respectively. The black bars in panel B indicate the presence of each characteristic scored with presence/absence or 1-0 sampling by any female during that stage.

Figure 2.7



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Chapter Three

Gelada infanticide risk

Introduction

Sexually selected male infanticide is a reproductive strategy in which males reap reproductive benefits by killing unrelated, dependent offspring (Hrdy, 1979; Hausfater & Hrdy, 1984). Although this is a widespread reproductive strategy found across species of birds, fish, and invertebrates, it is most common among mammals due to the dramatic imbalance in parental investment between males and females (Ebensperger, 1998). While females benefit from limiting their reproductive investment to high quality mates, males benefit by mating with as many females as quickly as possible (Trivers, 1972). Sexually selected male infanticide is defined by three requirements (Hrdy, 1979; Hausfater & Hrdy, 1984). First, males must only kill unrelated offspring. Second, the death of the infant must hasten the mother's return to fertility. In other words, a female must return to cyclicity earlier than if she had successfully weaned her infant. Third, this accelerated return to fertility must translate to increased reproductive benefits for the perpetrator (i.e., he must sire her next offspring). Across mammals, the most likely time for sexually selected male infanticide occurs when a new immigrant male takes over the dominant position in a group that contains unrelated, dependent offspring (van Schaik, 2000). Under such circumstances, the reproductive benefits to the infanticidal male are well established. However,

this extreme form of sexual coercion poses a salient threat to female reproductive fitness.

In chacma baboons (*Papio hamadryas ursinus*), a species with a high rate of infanticide, the replacement of the dominant male (hereafter, “takeover”) represents a greater source of physiological stress for the females at risk for infanticide (i.e., lactating females) than for females not at risk (i.e., cycling females) (Beehner, Bergman, Cheney, Seyfarth, & Whitten, 2005; Engh et al., 2006). In general, the stress response is an adaptation that has evolved to aid in the protection of an organism from both social and ecological challenges (Sapolsky, 2002). When confronted by a stressor, the mounting of a stress response primes the brain and body to respond to and dispose of that stressor. The stress response is mediated by the hypothalamic-pituitary-adrenal (HPA) axis which, when activated, results in a cascade of hormonal events. Briefly, when a stressor is perceived by the brain, the hypothalamus releases corticotrophin releasing hormone which acts on the anterior pituitary. In turn, the anterior pituitary releases adrenocorticotrophic hormone into the peripheral bloodstream which then acts on the adrenal gland to produce and release glucocorticoids. The glucocorticoids act throughout the body and in the brain, preparing the individual to overcome the stressor (Sapolsky, 2002). While this is a complex process, the majority of research on the stress response has focused on changes in glucocorticoids as a peripheral marker for stress (e.g., von der Ohe & Servheen, 2002).

In the case of the chacma baboons, the glucocorticoid response to takeovers in lactating females, but not cycling females, indicates that takeovers themselves are not stressful (if, say, they were associated with higher rates of aggression received by all females), but rather that takeovers may be associated with the psychological stress that accompanies infanticide risk. Such a stressor would only affect lactating females. Moreover, authors only report an elevated glucocorticoid response from lactating females after the arrival of a strange, immigrant male. A

similar takeover by a natal male (natal males are never known to commit infanticide) did not elicit a stress response from any females in the group (Beehner, et al., 2005).

So, what do chacma females do to counter the infanticide threat posed by new immigrant males? Chacma lactating females appear to form close ‘friendships’ with other group males (presumably the father of the offspring, e.g., Palombit, 1999; Weingrill, 2000; but not always, see Moscovice, Heesen, Di Fiore, Seyfarth, & Cheney, 2009; Moscovice et al., 2010) as ‘defensive alliances’ (Smuts, 1985) against infanticide. Although sample size precludes a direct analysis of whether such friendships lower the chances of infant injury and death (because most females form friendships with at least one male, it is difficult to assess the effectiveness of such a strategy, Palombit et al., 2000), it is clear from hormone data that such a strategy serves proximately to mitigate a female’s stress response. The presence of a male friend following a takeover decreases (Beehner et al., 2005) or completely suppresses (Engh et al., 2006) the glucocorticoid response in chacma females at risk for infanticide compared to those without male friends.

Similar to chacma baboons, sexually selected male infanticide is the leading cause of infant mortality in geladas (*Theropithecus gelada*); 43.7% of infants die following male takeovers (Beehner & Bergman, 2008). However, in contrast to the multi-male groups that occur in chacma baboons, geladas live in reproductive units (hereafter, “unit”) that normally consist of a leader male, 1-12 adult females and dependent young (e.g., Dunbar, 1984). These units form the base tier for geladas’ fission-fusion, multi-level societies which lead to strong inter-unit associations. As a result of the gelada social system, all known takeovers in geladas are by unfamiliar, unrelated males that come from all-male bachelor groups. Thus, in contrast with the patterns described for chacma baboons, all males should benefit by committing infanticide as

they start their tenure as leader, making it a plausible risk in every takeover.

The gelada social system may also allow for the use of defensive alliances as a counterstrategy to infanticide. Although geladas often live in units with only one male, approximately one third of units retain a subordinate follower male. The follower male can be either the deposed leader (generally the father of any dependent infants in the unit at the time of takeover) or a new male that enters the unit during the takeover process (Dunbar, 1984; Snyder-Mackler, Alberts, & Bergman, 2012). In cases of a deposed leader becoming a follower, we would expect that both the mothers and the deposed leader should work to protect dependent infants following a takeover. Further, if they do form defensive alliances, the relationship may be equivalent to the friendships reported for chacma baboons and thus mitigate the mother's stress response.

Here we examined infanticide risk, and the perception of that risk, in a wild population of geladas living in the Simien Mountains National Park of Ethiopia. We had two separate aims. Our first aim was to identify the time period during which infanticide is likely to occur and what demographic factors may contribute to infanticide risk following a takeover. We examined the potential effects of infant age, father presence, and unit size on the rates of infanticide. We hypothesized that younger infants would be at higher risk of infanticide than older infants due to the increased gain in time by the infanticidal male. Father presence (i.e., the deposed leader male remaining in the unit) was hypothesized to decrease infanticide risk due to potential defensive alliances with the mother. Additionally, we hypothesized that infanticide risk would be lower in large units than in small units because male reproductive success is overall higher in larger units (Snyder-Mackler et al., 2012) and, thus, the male may not benefit as much from committing infanticide in a larger unit. Our second aim was to investigate whether gelada females could

perceive their individual risks of infanticide. Our overall hypothesis was that females at higher risk for infanticide would exhibit a higher physiological stress response following takeovers than those at lower or no risk. We had two specific predictions for this hypothesis. First, in accordance with the chacma findings, we predicted that females at risk for infanticide (i.e., lactating females) would exhibit a greater physiological stress response (i.e., higher fecal glucocorticoids) to male takeovers than those not at risk (i.e., cycling females). Second, we predicted that the physiological stress response within lactating females would increase as infanticide risk increased.

Methods

Study site and species

We collected observational and physiological data from a wild population of geladas living in the Simien Mountains National Park in Ethiopia. Our observational data come from 109 females across 21 units over a 5-year period. For the hormone analyses, we utilized data from 81 of these females (across 15 units) that (1) experienced a takeover and (2) had sufficient hormone sampling both in the wake of the takeover (i.e., in the 2 months following the takeover) as well as outside of the takeover (i.e., well before or after the unstable period surrounding the takeover itself). These 15 units experienced a total of 19 takeovers. All females included in the observational versus hormonal data sets and fecal sampling are summarized in Table 3.1. All the research conducted in this study was entirely non-invasive and was approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan and adhered to the laws and guidelines of Ethiopia.

Observational data collection

We have complete demographic data for all females across the study period. These data included all births, deaths, takeovers, and changes in female reproductive state (see Chapter 2 for specific methods). Female reproductive state (i.e., cycling, pregnant, or lactating) was determined based on sexual swellings combined with the presence/absence of an infant. Hormonal data were later used to confirm any suspected pregnancies or the resumption of cycling (see below). We assigned females to one of the three reproductive states at the time of takeover, regardless of whether females changed state following the takeover (i.e., due to infanticide). Additionally, we recorded any changes in the number of adult (i.e., reached menarche) females in a unit, and we assigned parity for all females based on (1) known or assigned maternity to other infants (multiparous females); (2) the presence of “button” nipples prior to her infant’s birth (primiparous females); and (3) the continued presence of button nipples (nulliparous females).

Across the study period we have adequate data from 49 cases of lactation (from 39 individual females) and 104 cases of cycling (from 76 females) across the 20 takeovers. Sixteen females appear more than once across the two reproductive conditions (i.e., they were lactating after 1-3 takeovers and cycling after 1-2, no female was included more than four times). See Table 3.1 for a summary of females.

Of 49 lactational periods, we have exact dates of birth (DOB) for 35 of the infants and estimated dates (+/- 6 mos) for the other 14 infants. As previously used by Beehner and Bergman (2008), the DOBs for the remaining infants were estimated based on a combination of photographs of gelada infants with known ages and published descriptions of known ages of gelada infants (Dunbar & Dunbar, 1975). Considering that takeovers can take several days from start to finish, we assigned the date of takeover as the first day that the signs of a takeover were

observed. Exact dates of takeovers are known for 17 of 20 takeovers. For the remaining 3 takeovers, the units were not seen on the day of takeover. In these cases, the takeover date was conservatively estimated as the first potential date of takeover, i.e., the first day we did not see the unit. Following a given takeover we closely monitored the group for any injuries, deaths, disappearances, and changes in female reproductive state. In accordance with Beehner and Bergman (2008), all disappearances of dependent infants were considered deaths, as such infants are highly unlikely to survive without their mothers.

Hormone collection, processing, and analysis

Following takeovers, units were followed as closely as possible for the first month to monitor for demographic changes. Prior to 2009, fecal samples were opportunistically collected from females following a takeover. From 2009-2011, we aimed to get one sample/day for the first week, one sample/week for the next 3 weeks, and one sample/month for the next 11 months. Here we use data from 25 cases of lactation (23 females) and 70 cases of cycling (65 females). Seven females are represented in both reproductive categories in separate takeovers. Hormones were extracted from feces in the field, stored, and assayed for fecal glucocorticoids (fGC) in the lab using the methods described previously (Beehner & Whitten, 2004; Beehner & McCann, 2008, see Chapter 2 methods for collection details). Briefly, to measure fGCs, we used a double-antibody, corticosterone 125I RIA kit (MP Biomedicals, Orangeburg, NY). The primary antibody in this kit cross-reacts 100% with corticosterone, 0.34% with desoxycorticosterone, 0.1% with testosterone, 0.05% with cortisol, 0.03% with aldosterone, and 0.02% with progesterone. Prior to radioimmunoassay (RIA), all samples were incubated at room temperature for 1 h, and an aliquot of each sample was reconstituted in working buffer. Internal controls were run in every assay and consisted of three pooled fecal samples and two

immunoassay controls provided with the RIA kit. All standards were run in triplicate, all controls and samples were run in duplicate, and mean concentrations are expressed as ng/g. Because steroid hormones have a gut passage time of approximately 24 hours in geladas (Beehner and McCann, 2008), all fecal estradiol hormone values are assigned a date one day prior to fecal collection for all analyses.

Data analyses

All statistical analyses were conducted using RStudio (Version 0.94.110). Due to small sample sizes within categories, all cases (i.e., all females in all takeovers) were included separately. We acknowledge that this creates the problem of pseudo-replication in our data analyses and this should be considered when interpreting the results.

First, we sought to establish the time period and demographic factors that influence the infanticide risk. To identify the time period in which infanticides were observed in our population we used only infants in units with known dates of takeover and known dates of infanticide (N = 24 infants in 9 takeovers with known dates, N = 15 infanticides). We then examined how infant age, father presence (as a follower), and unit size predicted the proportion of infants killed following a takeover using all lactating females (N = 49). We included maternal parity as an additional potential predictor of infanticide risk as a proxy for female age. We used logistic regression to examine which factors predicted infanticide following takeover and used the Likelihood ratio test (-2 log likelihood) to select the best model.

Second, we examined whether females perceive their risk of infanticide by examining their hormonal response to the takeovers. Hormonal data were excluded from this analysis for three reasons. First, when a female's infant was killed, only data collected *before* the infant's death were included in analyses. Second, because infants older than 1.26 years are no longer

considered to be vulnerable to infanticide (i.e., this is the age when infanticide would no longer accelerate a female's return to fertility; Beehner and Bergman, 2008), these infants and their mothers were excluded from analyses. Third, pregnant females were not included in any analyses because the majority of pregnant females miscarried immediately after takeovers (see Chapter 4).

To control for individual differences in fGCs, we used each female's percent change from baseline as our dependent variable (as in van Anders, Brotto, Farrell, & Yule, 2009). Each individual's baseline (B) was used for each sample (S) in the following way to calculate percent change: $(S-B)/B*100$. Because takeovers are unpredictable we could not focus on collecting sufficient baseline data in the months leading up to a takeover. We therefore calculated "statistical baselines" from all samples collected across the 5-year period for each individual. Samples were excluded from the baseline calculation if they were: (1) outliers from the population (identified by Grubbs outlier test: 2 samples), (2) collected during a known pregnancy (because pregnancy can lead to hypercortisolism ; e.g., Mastorakos & Ilias, 2003), or (3) collected within 3 months following a takeover. therefore we looked only at samples taken from cycling and lactating periods to calculate individual baselines. To avoid interference from female response to takeover, we only included samples collected outside of the 3 months after a takeover. We had a mean of 13.94 samples per female (ranging from 2-72).

Perception of infanticide risk was first compared between lactating and cycling females using each individual mean percent change in fGC. Subsequently, within lactating females only, perception of infanticide risk was examined using Spearman's correlation between each individual's risk factor for infanticide and mean percent change in fGC in the post-takeover period. Here, risk factor is defined as the odds ratio: the probability of infanticide divided by the probability of infant survival. A risk factor over 1 would indicate a greater chance of infanticide

compared to infant survival, and a risk factor under 1 would indicate higher chance of survival. Individual risk factors for the subset of females with fGC samples were predicted using the full best fit logistic regression model from above based on the infant age and group size for that female.

Results

Determining infanticide risk

The infant survival curve following male takeovers indicates that all infanticides occurred within the first 7 weeks following a takeover (Figure 3.1). Infant age in days had a significant effect on infanticide occurrence (Likelihood ratio test, $\chi^2 = 8.85$, $df = 2$, $p = 0.003$) with younger infants having a higher risk than older infants. Unit size also had a significant effect on infanticide occurrence (Likelihood ratio test, $\chi^2 = 2.42$, $df = 2$, $p < 0.001$) with infants in smaller units being at higher risk than those in larger units. Neither maternal parity nor father presence affected the chances of an infant being killed and were not included in the model.

To facilitate interpretation, we then categorized infants into 3 age categories: 0-6 mos, 6-12 mos, and over 12 mos). Importantly, the oldest age group (>12 mos) only includes infants that theoretically should still be at risk for infanticide (i.e., up until 1.26 years of age as per Beehner & Bergman, 2008). We found 40% (12/30) infants under 6 months were killed, 16.7% (2/12) infants between 6 and 12 months, and no deaths when infants were more than 12 mos old (0/7). When unit size was divided into small (4-6 females) and large (7-9 females) units, we found 50% (9/18) of infants in small units were killed, whereas only 16.1% (5/31) of infants in large units were killed.

Perception of infanticide risk

Because all infanticides occurred within 7 weeks of the takeover, we restricted our

hormone analyses to the first two months after a takeover as our period of “infanticide risk”. Within this 2-month period following takeovers, lactating females exhibited significantly higher % change in fGCs from their baselines than did cycling females ($t(40.2) = -2.22, p = 0.03$; Figure 3.2). In other words, the fGCs of lactating females were significantly higher than baseline after takeovers, but the fGCs of cycling females were not. Note that *all* females experienced a takeover; the only difference between groups was their reproductive stage.

The highest risk factor in this population was 0.81 and the lowest was 0.01. A summary table of the risk factors for the different infant age groups and unit sizes are provided in Table 3.2. The overall correlation between maternal risk factor and maternal fGCs was not significant. However, when we break down these data into two categories: mothers whose infants are *not* killed and mothers whose infants *are* killed, we see a remarkable difference. In separate correlations, the Spearman’s rho revealed a significant positive relationship between risk factor and maternal fGC for mothers of infants that survived ($r_s[19]=0.48, p = 0.037$) and a significant negative relationship between risk factor and maternal fGC for mothers of infants that were eventually killed ($r_s[6]=0.99, p = 0.002$) (Figure 3.3). Put another way, only mothers who had elevated fGCs during the 2 months after a takeover had infants that survived. Further, only mothers of infants that survived actually had higher fGCs level post takeover than cycling females ($t(27.0) = -2.36, p = 0.03$, Figure 3.4). Mothers of infants that were eventually killed had fGC levels similar to those of cycling females prior to their infants’ deaths ($t(5.9) = -0.04, p = 0.67$) and the two lactating groups did not differ ($t(9.1) = 0.98, p = 0.35$).

Discussion

Our results for infanticide risk indicate that infanticide only occurs within the first 7 weeks following male takeovers. Infanticide risk is influenced by infant age and unit size such

that young infants in small groups are at the highest risk. These patterns were expected and support our hypotheses, but we were surprised that father presence did not influence infanticide outcomes. This suggests that defensive alliances are not a used, or usable, counterstrategy in geladas (see below for further discussion).

Our results for perception of infanticide risk indicate that females appear to perceive their differential risk. At the basic level, only females at risk of infanticide (i.e., lactating females) mount a stress response after male takeovers. In contrast, cycling females show no difference from baseline. This finding is in accordance with both our hypothesis and the basic patterns in fGCs in lactating versus cycling chacma baboons (Beehner et al., 2005; Engh et al., 2006). Further, using predicted risk that combines infant age and unit size into individual risk factors, we find that mothers of infants that survive produce higher fGCs as their risk for infanticide increases. In contrast, mothers of infants that are killed produced the opposite trend: as predicted risk increased, fGCs decreased.

The most remarkable finding of this study is that this acute stress response appears to promote infant survival. Despite the widely accepted assumption that the acute stress response is adaptive, there is very little evidence that directly links the acute stress response to actual fitness outcomes (Breuner, Patterson, & Hahn, 2008). Here, we demonstrate that lactating females whose infants survived takeovers by potentially infanticidal males were those that mounted a significant stress response after a male takeover. By contrast, females whose infants are eventually killed never produced a significant stress response. To our knowledge, this is the first study to demonstrate a link between an acute stress response and improved fitness in a wild primate.

These findings bear both comparable and contrasting patterns to those seen in chacma baboons. Lactating females in both geladas and chacma baboons produce higher physiological stress responses following a takeover than do cycling females (Beehner et al., 2005; Engh et al., 2006). However, the patterns within lactating females are strikingly different in the two species. Lactating chacma females with male friends have lower fGCs than females without friends (Beehner et al., 2005; Engh et al., 2006). These friendships are proposed to function as defensive alliances against the potentially infanticidal male (Palombit et al., 2000). Conversely, here we found no effect of father presence on infant survival. Why the difference? One potential reason may be that female chacma baboons have the opportunity to stay far away from the new dominant male and still remain in the social group (with their male friend for protection). Although quantitative data are not available on the spacing between the new dominant males and the females, chacma groups are more spread out in general compared to geladas. Following takeovers, chacma females with male friends tend to keep their distance from the new male, only keeping the company of their male friend (Beehner, pers.comm.) Geladas, on the other hand, remain in very close proximity to their entire units; females are rarely more than 40-50m away from the males, and even then, for only short periods of time. In addition, evidence from playback experiments in chacma baboons suggest that males would work to protect their female friends in aggressive interactions, particularly when the aggressor is an infanticidal male (Palombit, Seyfarth, & Cheney, 1997). Although we did not test this directly, if gelada fathers worked to protect their at-risk infants, we would expect their presence to influence infant survival, but it does not. More data would be required to directly test differential relationships between the fathers and lactating females following takeovers, but we currently have no evidence of male 'friends' in geladas.

Assuming that geladas cannot use defensive alliances as a successful counterstrategy to infanticide, and in light of the relationship between fGCs and infant survival, it is plausible that lactating female geladas actually need the acute stress response to save their infants. If females cannot avoid the leader male within the unit or leave the unit completely (female dispersal has never been observed; Dunbar, 1984), it is possible that the stress response is either a mechanism or side effect of an alternative counterstrategy. Currently, we do not know what the causal relationship is between higher stress and reduced infanticide risk; but, together, our data suggest that the stress response is part of a coordinated behavioral and physiological response to mitigate this risk

Table 3.1. Summary of females with observational and hormonal data with hormone sample sizes.

Reproductive state	Observational data cases	Subset with hormonal data	Baseline samples/female	Takeover samples/case
Lactating	49 (39 females)	25 (23 females)	17.9	5.9
Cycling	104 (76 females)	70 (65 females)	11.9	5.4

Table 3.2. Summary of risk ratios across infant age and unit size categories.

Infant age	Small units (4-6 females)	Large units (7-9 females)
	<i>Average risk ratio ± SD</i>	
< 6 months	0.68 ± 0.13	0.23 ± 0.08
6-12 months	0.23 ± 0.12	0.07 ± 0.04
>12 months	0.07 ± 0.02	0.03 ± 0.01

Figure 3.1

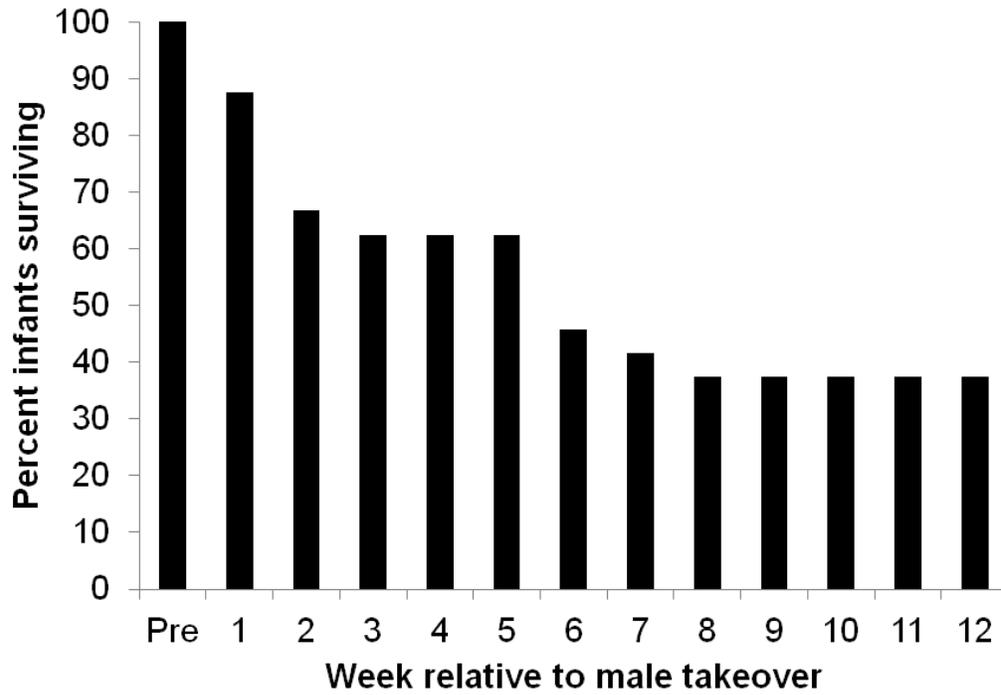


Figure 3.1. Infant survival post-takeover. For this population, no further cases of infanticide are reported after 7 weeks post-takeover.

Figure 3.2

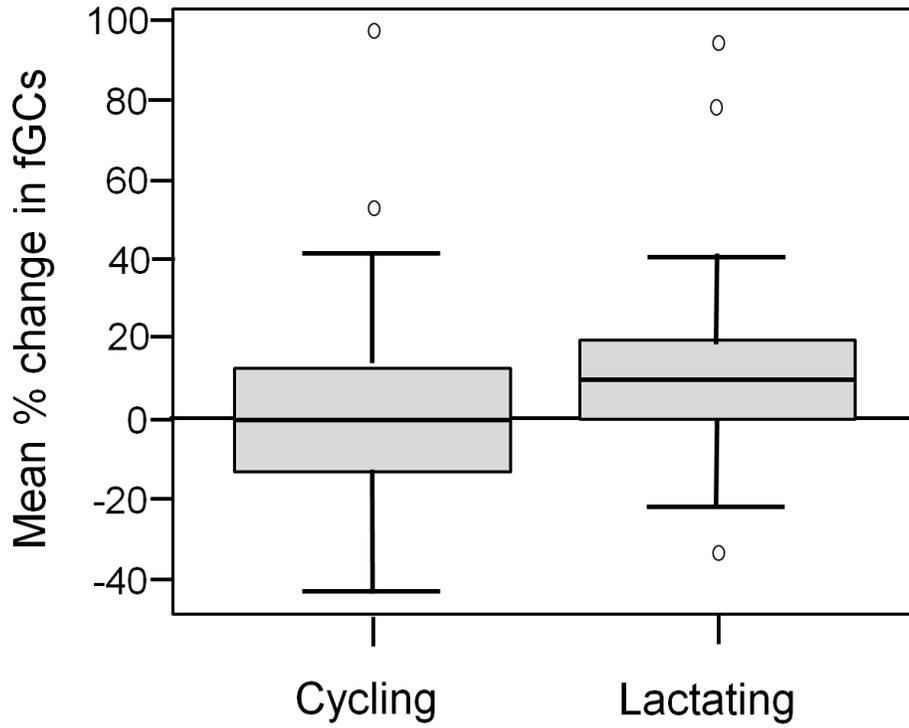


Figure 3.2. Mean % change in fGCs in cycling and lactating females following takeovers. Lactating females (i.e., females at risk of infanticide) exhibited a greater increase in fecal glucocorticoids than cycling females (i.e., females not at risk) across the first 2 months following takeovers ($p < 0.05$).

Figure 3.3

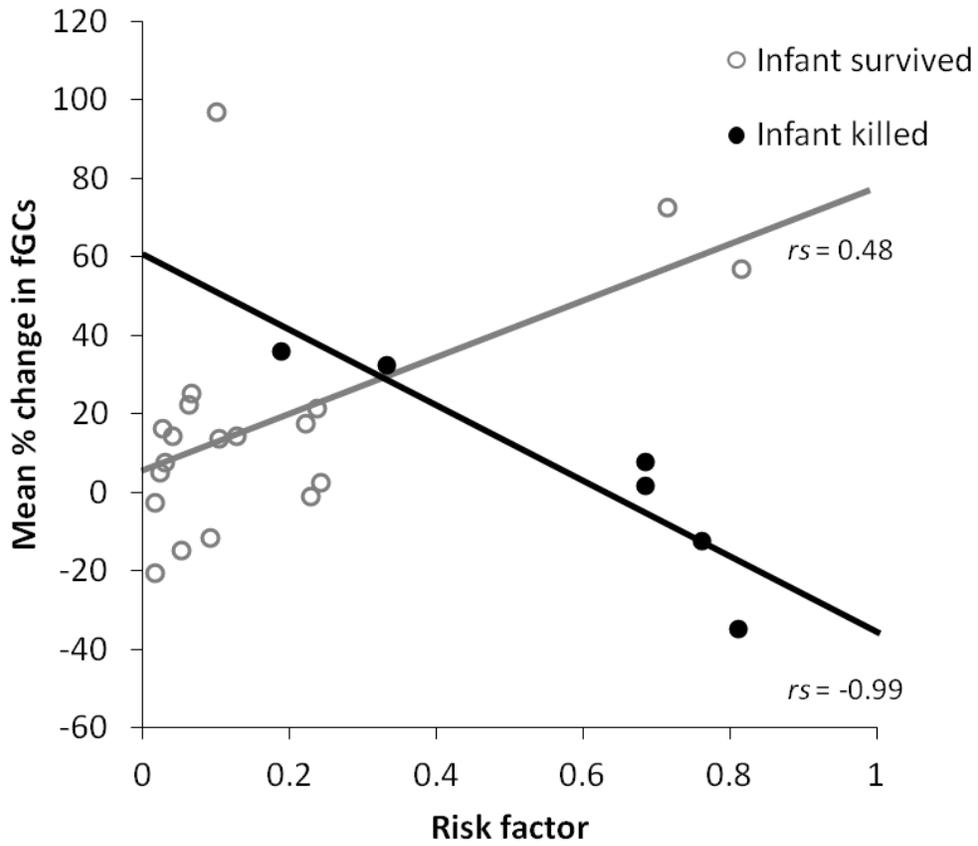


Figure 3.3. The relationship between female risk factor and mean % change in fGC. While there was a positive relationship between risk and fGCs in mothers of infants that survived (gray), the relationship was negative for mothers of infants that were killed (black).

Figure 3.4

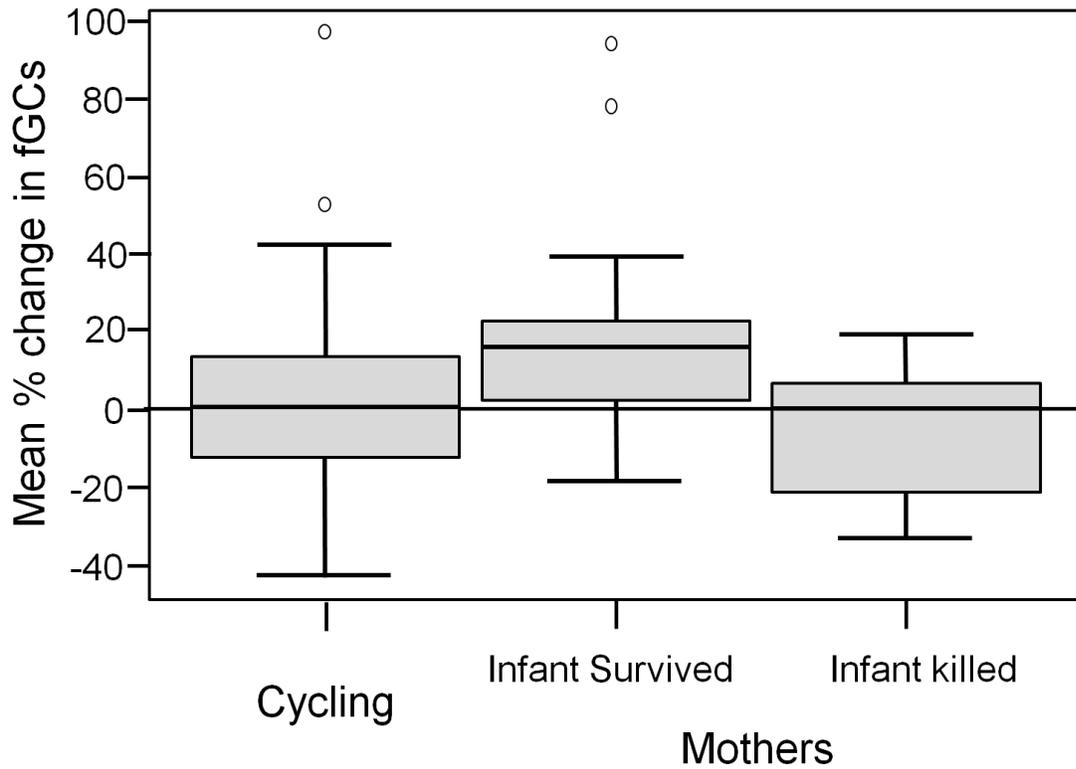


Figure 3.4. Mean % change in fGCs in cycling females, mothers of infants that survived, and mothers of infants that were killed following takeovers. Only lactating females whose infants survive produced glucocorticoid rises above cycling females ($p=0.01$). Note that only fecal samples prior to infant death in the “Infant killed” group were included in this analysis.

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Chapter Four

A Bruce effect in wild geladas

Introduction

Over half a century ago, biologist Hilda Margaret Bruce demonstrated that recently-pregnant female mice (*Mus musculus*) terminate pregnancies following exposure to unfamiliar males (“Bruce effect”)(Bruce, 1959; Bruce, 1960; Parkes & Bruce, 1961). Since these initial experiments, the Bruce effect has been experimentally confirmed across a multitude of rodent taxa (and domestic horses: Bartos, Bartosova, Pluhacek, & Sindelarova, 2011) under exclusively captive conditions (Becker & Hurst, 2009; Pillay & Kinahan, 2009; reviewed in Labov, 1981). However, despite the bounty of support for the Bruce effect in the laboratory, it remains unknown whether it occurs under natural conditions. At least two experimental studies conducted on wild rodents failed to find a Bruce effect (de la Maza, Wolff, & Lindsey, 1999; Mahady & Wolff, 2002), and all other reports of male-induced pregnancy termination from wild populations have been based on indirect (i.e., a drop in the birthrate: rodents: Mallory & Clulow, 1977; lions: Bertram, 1975; wild horses: Berger, 1983) or anecdotal evidence (i.e., direct observations of several aborted fetuses following the arrival of new males in Hanuman langurs: Agoramoorthy & Mohnot, 1988; Agoramoorthy, Mohnot, Sommer, & Srivastava, 1988; hamadryas baboons: Colmenares & Gomendio, 1988; yellow baboons: Pereira, 1983; and

geladas: Mori & Dunbar, 1985). The most comprehensive study to demonstrate a Bruce effect in a non-captive setting was conducted on wild (feral) horses relying on visual observations of pregnancy and estrus to ascertain fetal loss (Berger, 1983). This conspicuous dearth of direct support has led some to propose that the Bruce effect is nothing more than a laboratory artifact with no functional explanation (e.g., Wolff, 2003).

Certainly, the Bruce effect has been a conundrum to biologists since its discovery, as many (including Darwin, 1971) have pondered the evolutionary reasons why a female would terminate a pregnancy. The leading adaptive hypothesis is that it evolved as a counter-tactic for females whose offspring would be susceptible to infanticide after the arrival of a new male (Schwagmeyer, 1979; Hrdy, 1979). Under certain conditions, it may be less costly for a female to terminate a pregnancy than to waste investment on otherwise “doomed” offspring. However, no captive or wild studies have demonstrated a fitness advantage for females that terminate pregnancies in response to novel males.

Here, we use 5 years of demographic and hormone data to test for a Bruce effect in a wild primate population with a high risk of infanticide – the gelada (*Theropithecus gelada*). Anecdotal accounts on abortions following male replacements (Mori & Dunbar, 1985; Beehner & Bergman, 2008) prompted us to investigate whether female geladas exhibit a Bruce effect, and if so, whether such an effect is adaptive.

Geladas are terrestrial, Old World monkeys that live in polygynous, matrilineal, one-male units (“groups”) comprising one dominant male and 1-12 females (Dunbar, 1984). Reproductive success for dominant males is contingent upon maintaining reproductive control over the group. Threats to dominant males come from bachelor males residing in all-male groups. Once a dominant male is ousted and replaced by a bachelor, the bachelor male gains reproductive access

to all of the group's females and often kills any dependent offspring sired by his predecessor (Mori & Dunbar, 1985; Beehner & Bergman, 2008). As non-seasonal breeders with prolonged interbirth intervals (Beehner & Bergman, 2008) and high paternity-certainty (Bergman, Snyder-Mackler, & Alberts, 2011), infanticide is presumably an adaptive strategy for gelada males – it causes (previously) lactating females to resume cycling almost immediately, thus accelerating the next conception by the infanticidal male and significantly reducing the interbirth interval (Beehner & Bergman, 2008). Following male replacement, 43.8% of infants are lost to suspected infanticide by males (Beehner & Bergman, 2008). Given the high frequency of infanticide in geladas, a Bruce effect could be adaptive for pregnant females after the dominant male is replaced if it accelerates conception with the new male and minimizes the interbirth interval between successful offspring.

Methods

To test for a Bruce effect, we collected demographic and behavioral data from a wild population of geladas living in the Simien Mountains National Park, Ethiopia. Our study population comprised 110 females across 21 groups over a 5-year period (Jan 2006 - Jun 2011). First, we used demographic data to examine the pattern of births relative to male replacements (N=28 replacements). Specifically, we examined the number of births during the 6 months following a male replacement for each group. Assuming a 183-day gestation (± 4.8 days, N=16 females), any infants born during this period would presumably have been sired by the predecessor (i.e., candidates for a Bruce effect). To control for seasonality in male replacements and/or births, we paired each group that experienced a male replacement (“replacement group”) with a comparable group that did not experience one (“control group”). We selected control groups that: (1) had not experienced a male replacement during the previous year, (2) had not

already been used as a control group, and (3) had a nearly-equivalent (± 2) number of females as replacement groups. When more than one choice was available (N=4 cases), we conservatively chose the control group with fewer females. We then compared the number of births for replacement (28 male replacements, 140 females) and control groups (0 male replacements, 136 females) across the same period of time.

Results

Consistent with a Bruce effect, replacement groups exhibited a strikingly different pattern than control groups (Figure 4.1). During the 6 months before male replacements, paired groups exhibited no difference in the number of births (Wilcoxon signed-ranks test: $Z=-0.43$, $p=0.67$). However, during the 6 months after male replacements, replacement groups exhibited significantly fewer births than control groups (Wilcoxon signed-ranks test: $Z=-3.56$, $p<0.001$). Indeed, only 2 infants sired by the predecessor were born after male replacements (compared to 36 infants born in control groups). During the 7-12 months after male replacements, replacement groups exhibited a two-fold increase in births compared to control groups (Wilcoxon signed-ranks test: $Z=-2.65$, $p<0.01$).

Although these data are compelling, they provide only indirect evidence for a Bruce effect. To directly determine whether this reduction in births was due to pregnancy termination, we utilized fecal hormone data collected from specific females. Daily or weekly hormone sampling from all 110 females was not feasible. Therefore, we sampled females at different frequencies (daily, weekly, or monthly) based on their likelihood of being or getting pregnant during the study (Table 4.1). Steroid hormones (i.e., estradiol metabolites) were extracted from feces in the field, preserved using a solid-phase extraction cartridge, stored at sub-zero

temperatures until analysis, and assayed for 17β -estradiol (E2) using a radioimmunoassay (RIA) kit produced by MP Biomedicals (see Appendix for details).

We used estrogen metabolite profiles to identify all pregnancies, including those that did not result in a live birth (“pregnancy failures”). To do this, we used samples from females with daily sampling to establish normative fE levels for the non-perioovulatory period (mean fE=4.98 \pm 0.69 ng/g, N=7) and the perioovulatory period (mean fE=21.45 \pm 4.95 ng/g, N=7). We then assigned pregnancy based on a steady rise in fecal estrogen (fE) above perioovulatory levels (Beehner, Nguyen, Wango, Alberts, & Altmann, 2006). For females without daily hormone samples, a pregnancy was assigned if two fE values across more than 5 days were above perioovulatory levels. A pregnancy failure was assigned if no live birth resulted from a hormonally-determined pregnancy. One pregnant female (and her group) disappeared for 3 weeks around the expected time of birth and reappeared with no infant. We do not know if she aborted or lost the infant shortly after birth. Because this case was *not* associated with a male replacement, we conservatively included it as the only pregnancy failure in the background rate. After identifying all pregnancy failures, we then examined the timing of failures relative to male replacements (N=9 replacements, for this subset of data) to determine if the rate of failure during the month after male replacements was higher than the background rate.

The hormone data unambiguously confirm that females terminated pregnancies after male replacements (Figures 4.2 and 4.3). Of 60 hormonally-determined pregnancies, only 9 ended in pregnancy failure, with 8 of these occurring during the 2 weeks after a male replacement (range: 3-13 days post-replacement). These 8 failures occurred in 8 different females across 5 male replacements. The pregnancy failure rate during the 2 weeks after male replacements (80%, or 8 failures/10 pregnancies) was significantly higher than the background

rate (2%, or 1 failure/50 pregnancies; binomial test: $p < 0.001$). In one male replacement, the new male cleaved off a “daughter” group from a “parent” group (i.e., a group fission). The pregnancy failure after the fission occurred in the parent group (still led by the presumed sire), not the daughter group. Therefore, it is unclear whether this case represents a Bruce effect (i.e., male-induced) or just a pregnancy failure. Results do not change if we place this failure in the background category (binomial test: $p < 0.001$).

To establish the timing of pregnancy failures, we used fE values to estimate the day of conception. Fecal E data from successful pregnancies indicated that pregnancy fE is indistinguishable from non-pregnant fE until the third week of pregnancy for this species (N=11 pregnancies with weekly sampling) – a result also found for another closely-related species (Beehner, Nguyen, et al., 2006). Therefore, for all pregnancy failures, we assigned conception as 21 days before the initial fE rise above periovulatory levels. Based on these estimates, at the time of abortion, one female was in the first trimester (gestation day 54), six females were in the second trimester (gestation days 76-116), and one female was in the third trimester (gestation day 151), suggesting that a Bruce effect in geladas may be possible at any stage of gestation (see Table 4.2). In only 2 cases, did we observe the aborted fetus (Table 4.2). For the other 6 cases (particularly late 2nd and 3rd trimester pregnancy terminations), we expect that the fetus was expelled and lost during the night on the sleeping cliffs.

An analysis of interbirth intervals (IBIs) indicates that a Bruce effect may have fitness benefits for gelada females (Figure 4.4). Note that all IBIs calculated below refer to the time period between a *surviving* infant and the subsequent infant. Outside the context of male replacements, the mean IBI for this population was 2.37 years (± 0.51 SD; N=37 IBIs). Following male replacements, the mean IBI for females that aborted was 2.65 years (± 0.54 SD; N=6 IBIs) –

a non-significant difference from the population mean (Mann-Whitney test: $Z=-1.21$, $p=0.23$). By contrast, females that lost their infants to infanticide had an IBI of 3.62 years (± 0.82 SD; $N=3$). To increase the sample size for statistical comparisons, we added IBIs from females that lost their infants due to other causes (i.e., predation, illness: $IBI=3.81\pm 0.44$ years; $N=2$), resulting in a mean IBI of 3.70 years (± 0.63 SD; $N=5$). This IBI after the death of an infant is significantly longer than the population mean (Mann-Whitney test: $Z=-3.24$, $p=0.001$, Figure 4.4) or the IBI following abortions (Mann-Whitney test: $Z=-2.19$, $p=0.028$, Figure 4.4). Correcting for the odds of infanticide (43.8%), the average IBI for females that do not abort is 2.95 years – still higher than either the population mean or the IBI following abortion. Further, because male tenure as the dominant male in a group (~ 3 years, Beehner & Bergman, 2008) is longer than the mean IBI for this population, females that time conceptions with male replacements are likely to be weaning (or have weaned) those infants by the next male replacement.

Discussion

What might the proximate mechanism be for a Bruce effect in geladas? In rodents, the presence of strange males can trigger a Bruce effect via pheromonally-induced gonadotropin release in the female, causing decreased prolactin secretion (Parkes & Bruce, 1961; Dominic, 1966; Milligan, 1976). Additionally or alternatively, a Bruce effect could also be triggered by heightened levels of physiological stress associated with new males (e.g., increased levels of aggression (Pereira, 1983), elevated infanticide risk) or other stressful situations (e.g., elevated predation risk, Becker & Hurst, 2008). The latter mechanism would suggest that a Bruce effect is part of a more general female strategy to curtail investment in offspring that may not survive. At present, because abortions occurred almost immediately after male replacements, our hormonal

and behavioral data were not sufficiently fine-grained across these critical few days to test a stress mechanism.

Taken together, the demographic and hormonal data suggest that the Bruce effect is not only a *frequent* counterstrategy to infanticide employed by pregnant geladas (utilized by 80% of pregnant females following male replacements), but also an *advantageous* one. Under conditions where infant survival is minimal due to susceptibility to infanticide, natural selection should favor females that retain the ability to terminate pregnancies prior to a hefty investment in gestation and lactation. Pregnancy termination by females (in particular, a Bruce effect) may be a facultative response that allows females to maximize their reproductive output in a rapidly changing social (or physical: Beehner, Onderdonk, Alberts, & Altmann, 2006) environment.

It is unclear how widespread the Bruce effect may be across mammals. First, data are scarce. Demonstrating a Bruce effect in a wild population requires frequent hormone sampling from females both before and after an unpredictable event (i.e., a male replacement). The effect may therefore be difficult to detect, and possibly more widespread than we realize.

Second, a Bruce effect may not constitute an adaptive counter-tactic to infanticide for all female mammals. As previously reported (Pillay & Kinahan, 2009), only species characterized by polygyny or monogamy, high paternity certainty, and high infanticidal risk should be prime candidates for a Bruce effect. However, equally important, a female's subsequent offspring must be less susceptible to infanticide than her current one. In other words, a female should only exhibit a Bruce effect if there is some certainty that her next offspring will be spared the same fate. The Reproductive Suppression Model (Wasser & Barash, 1983) suggests that, when future reproductive conditions are likely to be better than present ones, females should suppress reproduction; and when future conditions are unlikely to improve, females should continue with

current reproduction regardless of cost. In geladas, male tenure as the dominant male (~3 years) is roughly equivalent to the IBI (2.62 years). Therefore, females that immediately conceive with an incoming male have a high probability that the new sire will remain dominant until the offspring is weaned. Species without such assurance should not exhibit a Bruce effect. For example, chacma baboons (*Papio ursinus*) – also characterized by polygyny, high paternity certainty, and high infanticide rates – have a much shorter male tenure (~6 months, Hamilton & Bulger, 1990) than IBI (~2 years, Cheney et al., 2004). Under such circumstances, females that conceive with a dominant male have a very *low* probability that the sire will remain dominant through weaning (indeed, a chacma female is likely to see 3-4 different males across her 2-year lactational amenorrhea). When prospects for future offspring survival are no better than those for the current offspring, a Bruce effect is not an effective strategy. Testing whether the Reproductive Suppression Model explains the distribution of a Bruce effect across mammals must await further data from natural populations.

Table 4.1. Fecal hormone sample schedule and collection rates.

	Groups	Females ¹	Fecal Samples	Sample Rate
High-coverage	5	10	331	1 Sample/1.9 days
Mid-coverage	14	62	670	1 Sample/7.6 days
Low-coverage	18	88	1000	1 Sample/29.2 days
Total	20	106²	2001	

¹Number of females in each category does not include all females in the group.

²High-coverage females were selected based on probability of becoming pregnant.

²Females are represented by more than one "coverage" schedule, therefore the total number of females does not add up to the sum of each category.

Table 4.2. Females with hormonally-determined pregnancies at the time of male replacement, pregnancy outcomes, and female demographics.

Female ID	Pregnancy outcome	Trimester ¹	Observed signs ²	Group size ³	Rank ⁴	Parity ⁵
Hop	failure	1	-	9	mid	multiparous
Cas	failure	2	fetus	2	low	primiparous
Coo	failure	2	-	6	high	multiparous
Cor	failure	2	blood	6	high	multiparous
Waf	failure	2	fetus	6	high	nulliparous
Cal	failure	2	blood	6	low	multiparous
Tub	failure	2	-	6	low	multiparous
Cee	failure	3	-	6	mid	nulliparous
Tru	success	2	N/A	6	low	multiparous
Mab	success	3	N/A	8	low	multiparous

¹Trimester the female was in when dominant male was replaced by new male

²Any visual signs recorded by observers that indicate fetal loss

³Number of adult (i.e., post-menarche) females in the group

⁴Females in each group were split into thirds and assigned high, mid, and low categories

⁵We do not know ages for these females, so parity is presented as an age estimate

Figure 4.1



Figure 4.1. Number of births for groups with and without male replacement. Sum of births from groups with a male replacement (light grey) as compared to a similar-sized group at the same time that did not experience a male replacement (dark grey). Paired groups were compared (Wilcoxon signed-ranks test) 0-6 months before male replacement, 0-6 months after male replacement, and 7-12 months after male replacement.

Figure 4.2

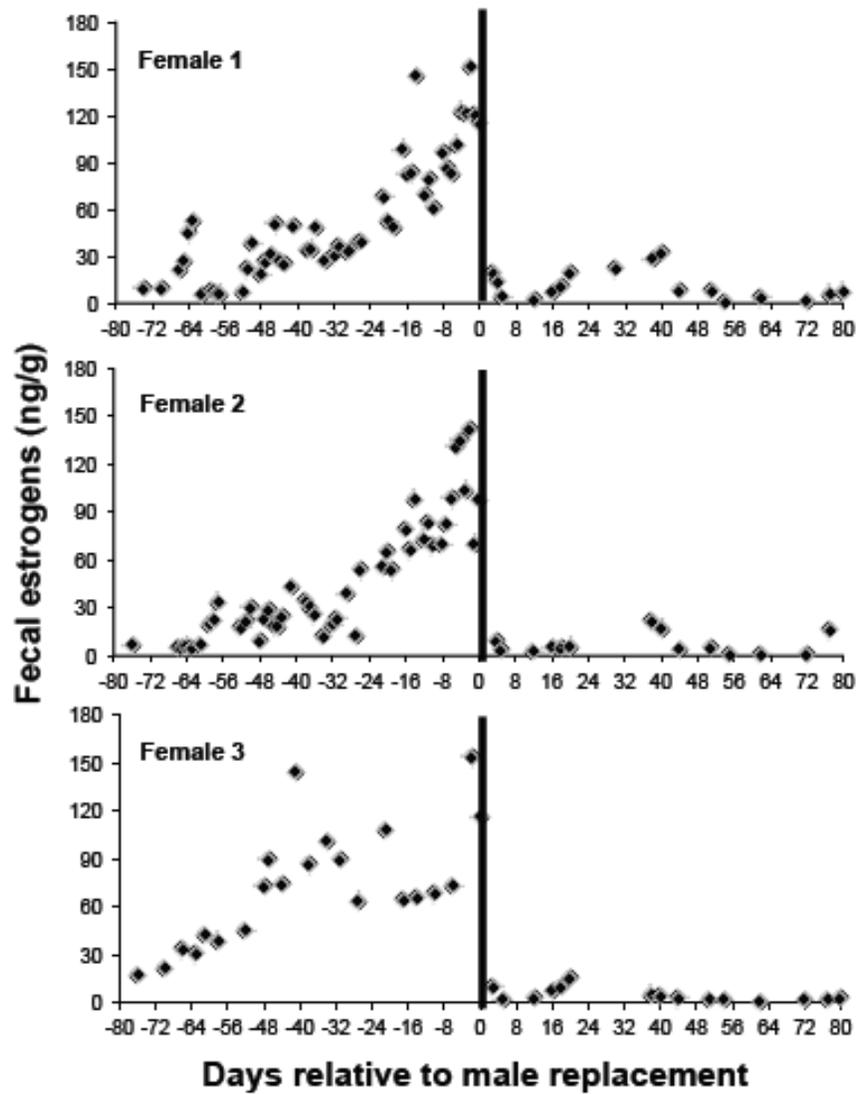


Figure 4.2. Hormonally-determined pregnancy failures. Hormone (fE, ng/g) profiles of three representative females that aborted following male replacement. Dark vertical lines indicate the time of male replacement.

Figure 4.3

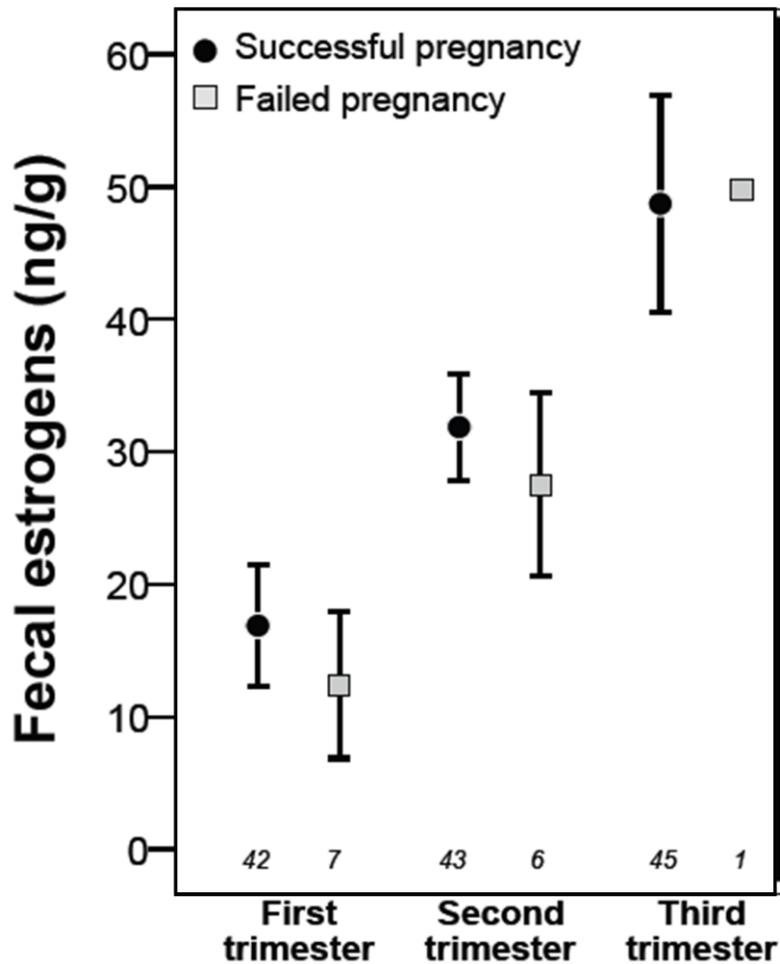


Figure 4.3. Composite comparison between hormone (fE, ng/g) profiles of successful (“success”) and aborted (“failure”) pregnancies across each trimester. Estrogens of successful and aborted pregnancies show no significant differences prior to male replacement. After male replacement (vertical line), aborted pregnancy estrogen profiles drop well below pregnancy levels. Sample size (number of pregnancies) is indicated in italics above the x-axis. One female that aborted was not included in the figure because we were unable to obtain a hormone sample from her immediately post male-replacement (she is known to have aborted because she expelled a poorly-developed fetus the day after a male replacement, Bechner & Bergman, 2008).

Figure 4.4

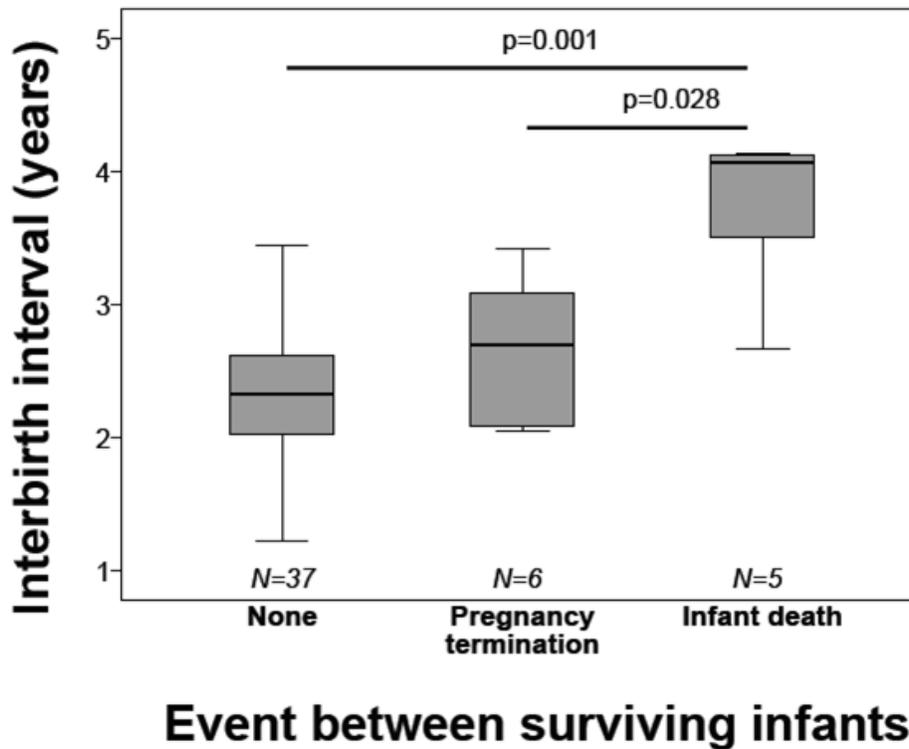


Figure 4.4. Interbirth intervals between successfully-weaned infants. Interbirth intervals (in years) for females experiencing different events between infants (none: two successive infants, pregnancy failure: one abortion between successive infants, infant death: one infant death between successive infants). Sample size (number of interbirth intervals) is indicated in italics above the x-axis.

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Appendix

Materials and Methods

Hormone collection, extraction, and analysis. Fecal samples were collected within minutes after deposition from positively identified individuals. Hormones were then extracted from feces using a method described previously (1). Specifically, the entire fecal sample was mixed thoroughly with a wooden spatula, and an aliquot of the mixed sample (~ 0.1 g wet feces) was placed in 3 ml of a MeOH/acetone solution (4:1). The solution was immediately homogenized for 1 min using a battery-powered vortexer (BioVortexer, BioSpec Products, Inc., Bartlesville, OK). Approximately 6-8 hours later, 2.5 ml of the fecal homogenate was filtered through a 0.2 µm polytetrafluoroethylene (PTFE) syringless filter (Whatman, Florham Park, NJ), and the filter was subsequently washed with an additional 1 ml of MeOH/acetone (4:1). We then added 7 ml of distilled water to the filtered homogenate, capped and mixed the solution, and loaded it onto a reverse-phase C18 solid-phase extraction cartridge (Sep-Pak Plus, Waters Corporation, Milford, MA). Prior to loading, Sep-Pak cartridges were prepped according the manufacturer's instructions (with 2 ml MeOH followed by 5 ml filtered water). After the sample was loaded, the cartridge was washed with 1 ml of a sodium azide solution (0.1%). All samples were stored on cartridges in separate sealed bags containing ~2 g of silica beads. Cartridges were stored at ambient temperatures for one week (to ensure adequate drying) after which all samples were stored at subzero temperatures (-10°C) until transported to the University of Michigan for analysis. In the laboratory, steroids were eluted from cartridges with 2.5 ml 100% MeOH and subsequently stored at -20°C until the time of assay.

All samples were assayed for 17 β -estradiol (E2) using a radioimmunoassay (RIA) kit produced by MP Biomedicals. Because commercially-available antibodies have been validated

only for a specific taxonomic group (i.e., humans for the antibody used here) and for a specific substrate (serum), adapting the antibody for use on gelada fecal hormones requires analytical validation (i.e., establishing parallelism, accuracy, sensitivity, and precision). Our validation demonstrated that the antibody was (1) parallel (a dose-response curve using serially-diluted samples was parallel to the standard curve), (2) accurate (accuracy tests where we spiked standards with a low sample found a mean recovery of 100.38% (N=6, $y=1.36x-2.44$, $R^2=0.99$)), (3) sensitive (lowest detection for our samples was 0.125 pg/tube (or 5 pg/ml)), and (4) precise (inter- and intra-assay CVs for a low and high sample were 15.42% and 14.24% (N=34) and 8.74% and 14.73% (N=10), respectively).

Prior to RIA, all samples were incubated at room temperature for one hour. Then, an aliquot of each sample was evaporated to dryness under nitrogen. Sample aliquots were determined such that hormone metabolite values were within the range of optimal precision of the assay. Kit protocols were followed except that all reagents were halved from the amount suggested by the manufacturer (a common technique employed by researchers measuring fecal steroids to maximize the use of each kit). Internal controls were run in every assay and consisted of a high (binding at 20%) and a low (binding at 80%) “pool” (a composite of many fecal samples). All standards were run in triplicate, all controls and samples were run in duplicate, and mean concentrations are expressed as ng per dry gram of fecal material (ng/g). The MP Biomedicals E2 antibody is known to have minor cross-reactivities with other estrogen metabolites (estrone: 20%; estriol: 1.5%; estradiol-17 α : 0.7%).

Chapter Five

Deceptive fertility in wild geladas

Introduction

Sexually selected infanticide by males is a threat to female reproductive fitness (e.g., Hrdy, 1979; Hausfater & Hrdy, 1984) that often occurs when a new male assumes the dominant position in a social group (see van Schaik & Janson, 2000 for review). When confronted by this threat, females face two potential strategic routes to protect their reproductive investment. They can either mitigate the costs by giving up on their current infant to save effort for future reproduction ('cut their losses') or try to prevent the cost by protecting their current infant. In primates, lactating females have several potential mitigative and preventative counterstrategies that they can employ to protect their reproductive effort against infanticide (see Chapter One for full discussion). The counterstrategies vary in the costs associated with them, even if they are 'successful' in protecting the life of the at-risk infant from infanticide. For example, prematurely weaning an infant (e.g., Colmenares & Gomendio, 1988) is a mitigative strategy that could potentially lead to infant death outside of infanticide (e.g., as a result of predation) or negative effects later in the infant's life (e.g., slower growth, lower reproductive success). In contrast, cheaper behavioral counterstrategies, such as maternal aggression or defensive alliances, have only been observed to delay, and not prevent, infanticide outside of rodents (e.g., hanuman langurs, Mohnot, 1971; gorillas, Watts, 1989; reviewed in Ebensperger, 1998). Thus, for female

primates that attempt to use these, the cost may be bodily harm to the mother and her allies in addition to the inevitable infant loss. Perhaps the cheapest effective counterstrategy available to primate mothers at risk for infanticide is deceptive fertility (Hrdy, 1974, 1979). A female exhibiting deceptive fertility will produce all outward signs of fertility in the absence of ovulation, including mating with the male. Here, a female reduces the male's incentive to kill her infant by providing the new male with the opportunity to mate with her, while she continues to invest in her current offspring. While this strategy is seen in a number of species with only behavioral indicators of fertility (e.g., hanuman langurs, *Presbytis entellus*, Hrdy, 1974; gorillas, *Gorilla gorilla*, Watts, 1989), it is more complicated for species with conspicuous morphological indicators such as sexual swellings.

Although some degree of sexual swelling is common among primates, exaggerated sexual swellings occur in only about 10% of all primate species (Dunbar, 2001) and are limited only to Old World monkeys and apes (Nunn, 1999). In the context of deceptive fertility, such sexual swellings may require more intensive physiological changes compared to only mating behavior. Evidence suggests that such sexual swellings are reliant upon circulating estrogen related to fertile cycles (e.g., Gesquiere, Wango, Alberts, & Altmann, 2007) and swellings generally reach peak turgescence at peak fertility (e.g., Dixson, 1983). However, sexual swellings have been observed outside of ovulation in a number of primate species during early pregnancy (e.g., sooty mangabeys, *Cercocebus torquatus atys*, Gordon, Gust, Busse, & Wilson, 1991; bonobos, *Pan Paniscus*, Heistermann, Mohle, Vervaecke, vanElsacker, & Hodges, 1996; chimpanzees, *Pan troglodytes*, Wallis, 1982; pigtail macaques, *Macaca nemestrina*, mandrills, *Mandrillus sphinx*, and drills, *M. leucophaeus*, Hadidian & Bernstein, 1979). In these cases, the deceptive swellings and associated copulations are thought to confuse paternity by extending a

female's mating over a longer period of time (e.g., van Schaik & Kappeler, 1997). In this sense, the deceptive swellings function as a counterstrategy to infanticide, but prior to the actual time period of infanticide risk.

Female hamadryas baboons (*Papio hamadryas*) produce sexual swellings earlier than expected during lactation when a new, potentially infanticidal, male replaces the male in their one male group (Colmenares & Gomendio, 1988; Zinner & Deschner, 2000). There is no hormonal evidence to confirm that these swellings were deceptive (i.e., outside of ovulation), but the mothers are observed to continue nursing their infants. Further, females that produce swellings sooner following these male replacements (hereafter, 'takeovers') do not have shorter interbirth intervals than females outside of takeovers, indicating that they do not return to a fertile state earlier than normal (Zinner & Deschner, 2000). Regardless, these swellings are proposed to function as a counterstrategy to infanticide because most of the infants of mothers that swell survive the takeover (Zinner & Deschner, 2000), but data thus far are insufficient to compare with the survival of females that do not swell.

As a potential counterstrategy to infanticide, it is clear that deceptive swellings and sexual behavior would have two adaptive benefits; (1) the act of copulation would decrease the incentive a male would have to kill the female's offspring; and (2) females may increase the probability of their infants' survival while avoiding the costs of being simultaneously pregnant and lactating. Mechanistically, the pathway may be more complicated. Lactation among mammals is generally characterized by very low levels of ovarian hormones (Johnson, 2007). If sexual swellings are indeed dependent on circulating estrogen at levels that are generally related to fertile cycles (e.g., Gesquiere et al., 2007), the mechanism that would allow females to produce sexual swellings while continuing to lactate needs to be addressed. There are two

potential issues with the female producing estrogen to an ovulatory level during lactation. First, due to the energetic expenses normally associated with lactation (Wade & Schneider, 1992), females may not be able to invest the energy into producing true ovulations during this period. Second, exogenous estrogen can suppress lactation (Johnson, 2007). Therefore, even if females can energetically return to fertile ovarian cycles, this could disrupt milk production as energy is redirected to the next potential reproductive event.

Another potential mechanism is suggested by research with laboratory mice. Numerous studies have examined the potential mechanisms by which males can influence female reproductive physiology in mice related to the Bruce effect (pregnancy block, Bruce, 1959) and the Vandenberg effect (acceleration female maturation, Vandenberg, 1967). Both phenomena have been clearly linked to female nasal exposure to male urine (Parkes & Bruce, 1962; deCatanzaro, Beaton, Khan, & Vella, 2006). In the case of the Vandenberg effect, male mice exposed to immature females produce higher concentrations of unconjugated urinary estradiol than isolated males and direct their urine towards the females (deCatanzaro, Khan, Berger, & Lewis, 2009). Recent data have clearly illustrated that urinary estradiol excreted by male mice can directly enter female circulation and arrive in the female reproductive tract (Guzzo, Jheon, Imtiaz, & Decatanzaro, 2012). Further, estradiol has been shown to enter the female system not only via nasal exposure, but also by dermal exposure (Guzzo et al., 2012). While these data come only from close-quartered laboratory rodents, corroborating evidence in human males show that adult men excrete high concentrations of unconjugated estradiol and testosterone in both facial and axillary perspiration (Muir et al., 2008). While these data are inconclusive outside of mice, female nasal or dermal absorption of male-excreted estrogens is an intriguing potential mechanism for deceptive swellings.

Here we examine both the mechanism and function of deceptive fertility as a counterstrategy to infanticide in geladas (*Theropithecus gelada*). Geladas live in reproductive units generally comprised of a leader male, 1-12 adult females, and dependent offspring (Dunbar & Dunbar, 1975). Sexually selected male infanticide is the leading cause of infant mortality in geladas, 43.7% of infants die following unit takeovers (Beehner & Bergman, 2008). Female geladas, like many Old World monkeys, produce sexual swellings around the time of ovulation (Dunbar & Dunbar, 1974). However, unlike other monkeys, gelada sexual swellings are situated around a patch of exposed skin on the chest. Geladas also have perineal swellings, but these swellings are much smaller and less variable in color and size than those of other species (e.g., Alvarez, 1973).

Although geladas were previously thought to produce sexual swellings only around the time of ovulation (Dunbar & Dunbar, 1974), they have recently been documented to both swell and copulate throughout the cycle (e.g., McCann, 1995; Chapter Two) and into pregnancy (Chapter Two). This flexibility in their sexual swellings and mating behavior may indicate flexibility in the hormonal determination of their swellings that goes beyond cycles and pregnancy. Further, field studies suggest that lactating female geladas return to a fertile state immediately following takeovers (Dunbar, 1980, Mori & Dunbar, 1985), but there is currently no evidence that they are deceptive fertility or that they function as a counterstrategy to infanticide. In terms of potential mechanisms for deceptive sexual swellings, recent evidence for the Bruce (Roberts, Lu, Bergman, & Beehner, 2012/Chapter Four) and the Vandenberg (Lu & Beehner, 2012) effects in geladas gives us a reason to explore female exposure to male estrogens as is seen in mice (Guzzo et al., 2012). The gelada social system provides an excellent system in which to test this mechanism in a wild species. The individuals within units remain relatively close to one

another throughout the day and sleep together on the cliffs at night. It is plausible that females are exposed to sufficient male-excreted estrogens from urine, or dermally through grooming or copulations, to stimulate the sexual swellings.

Here we test the hypothesis that lactating geladas use deceptive fertility as a counterstrategy to infanticide following takeovers. In order to examine both the mechanism and function of deceptive fertility as a counterstrategy, we formed and tested four separate predictions. Our first prediction was that undergoing a takeover during lactation affects the timing of the first postpartum swelling. We test this in two ways. First, we examine the timing of the first postpartum swelling relative to takeover. If takeovers trigger the swellings, we would expect that more females should begin swelling during the 2 months following takeovers than outside of takeovers. We specify the first 2 months because, as previously discussed, all known cases of infanticide occur within the first 2 months following takeovers (Chapter 3). Second, these swellings are expected to occur earlier for lactating females that undergo a takeover than for females outside of takeovers. Our second prediction was that the swellings produced immediately following takeovers are deceptive (i.e., not associated with ovulation). We tested this in two ways. First, if the accelerated return to outward fertility is not associated with accelerated conception, lactating females that go through a takeover should have similar interbirth intervals to females outside of takeovers (i.e., they should not get pregnant sooner because they do not actually ovulate sooner than females outside of takeovers). Second, if ovulation is not taking place, the females that swell following a takeover should not produce periovulatory levels of fecal estrogens. Our third prediction relates to the potential mechanism of these deceptive sexual swellings. If the swellings are separate from ovulatory cycles, we predicted that the females that produce sexual swellings are those being exposed to more urinary

estrogens from the new leader male than females that do not swell. Here, we use distance to the new leader male as a proxy for exposure and expect that females that produce swellings should remain closer to the new leader male than should females not producing swellings. Lastly, to explore the function of deceptive fertility as a counterstrategy to infanticide, our fourth prediction was that lactating females that exhibit deceptive fertility (i.e., sexual swellings and mating with the new male outside of ovulation) should have lower chances of infanticide.

Methods

Study site and subjects

This research was part of the long-term University of Michigan Gelada Research Project (UMGRP) that has been continuously monitoring a wild population of geladas in the Simien Mountains National Park (13° 15'N, 38° 00'E, elevation: 3250m a.s.l.) since 2006. Specifically, we utilize observational data from 109 females from 21 units, leading to the observation of 28 takeovers (2006-2011). We use hormonal data on a subset of these females: 63 females in 15 units. All individuals are individually recognized and fully habituated to observers on foot. All of our methods for this study were non-invasive, and all research was approved by both the Ethiopian Wildlife Conservation Authority and the University of Michigan University Committee for the Use and Care of Animals.

Observational data collection

Detailed demographic data are constantly collected for all target animals in our study population. These include records of all births, deaths, takeovers, and any changes in female reproductive state (see Chapter 2 for specific methods). For this analysis, we determined female reproductive state at the start date of their takeover such that a female lactating at the time of takeover was considered lactating even if her infant was eventually killed following the takeover.

Here, we determined female reproductive state by several means: (1) presence/absence of dependent young, (2) current sexual swellings, and (3) history of sexual swellings. We used behavioral data to ascertain whether or not a female mated during the post-takeover period as well as to examine spatial relationships between females and leader males. Behavioral observations were conducted in two ways: (1) 15-minute focal animal samples (Altmann, 1974) to record all social behaviors for focal individuals and (2) all occurrence records of copulations. Data on spatial relationships came from instantaneous distance samples that were collected in conjunction with the focal samples. We collected 3 samples of female distance to the leader male (at 5, 10, and 15 minutes) during each the focal. As in previous chapters, we followed units as closely as possible for the first month following a takeover to monitor for any demographic, reproductive, and behavioral changes.

Hormone data collection

Prior to 2009, fecal samples were opportunistically collected from females following a takeover. From 2009-2011, we aimed to get 1 sample per day for the first week, once a week for the next 3 weeks, and once a month for the next 11 months. Hormones were extracted from feces in the field, stored, and fecal estrogen metabolites (fE) were measured using radioimmunoassay (RIA) in the lab using methods described previously (Beehner & Whitten, 2004; Beehner & McCann, 2008, see Chapter 2 methods for extraction details).

For the RIA, we assayed all samples for estrogen metabolites using a commercially available kit (17 β -estradiol Double Antibody RIA kit, MP Biomedicals, see Roberts, et al., 2012 for validation). Prior to RIA, all samples were incubated at room temperature for one hour. Then, an aliquot of each sample was evaporated to dryness under nitrogen. Sample aliquots were determined such that hormone metabolite values were within the range of optimal precision of

the assay. Kit protocols were followed except that all reagents were halved from the amount suggested by the manufacturer (a common technique employed by researchers measuring fecal steroids to maximize the use of each kit). Internal controls were run in every assay and consisted of a high (binding at 20%) and a low (binding at 80%) “pool” (a composite of many fecal samples). All standards were run in triplicate, all controls and samples were run in duplicate, and mean concentrations are here expressed as ng per dry gram of fecal material (ng/g). The MP Biomedicals E2 antibody is known to have minor cross-reactivities with other estrogen metabolites (estrone: 20%; estriol: 1.5%; estradiol-17 α : 0.7%). Inter- and intra-assay CVs for a low and high sample were 15.42% and 14.24% (N=34) and 8.74% and 14.73% (N=10), respectively. Because steroid hormones have a gut passage time of approximately 24 hours in geladas (Beehner & McCann, 2008), all fecal estradiol hormone values are assigned to a date one day before the fecal collection for all analyses.

The effect of takeover on the timing of postpartum sexual swellings

The effect of takeover on the timing of postpartum swellings was examined in two ways. First, the timing of the first postpartum swelling was examined across all females for the month relative to the closest takeover. Here we included all cases of postpartum amenorrhea for females with known dates of the first postpartum sexual swelling and closest takeover (N = 133 cases across 92 females). Second, the length of postpartum amenorrhea (calculated as the time between the known date of birth and the first subsequent postpartum swelling) of females that experience a takeover while lactating (N = 31) was compared to the population mean (N = 22 females where a mean was taken if a female had more than one IBI outside of takeovers, Chapter Two).

Are these swellings deceptive?

To test whether the early postpartum swellings following takeovers leads to earlier conception of the next pregnancy (as per Zinner and Descner, 2000), we examined interbirth intervals (IBIs) that were calculated as the time between two consecutive births with exact known birth dates. We compared the IBIs for females that were lactating at the time of takeover (N = 23) to the population mean (N = 45, Chapter Two). Then we explored whether the early postpartum swellings were associated with hormonal signs of ovulation. We examined the fE values for all samples from females that swelled following takeovers (N = 12) during and around the period of the swellings and compared those to the periovulatory values seen in true ovarian cycles (N = 7, Chapter Two) and lactation outside of takeovers (N = 59).

Exploring mechanism

Although we do not currently have a method for measuring estrogen concentrations in gelada urine, we can examine patterns of proximity as a proxy for potential exposure to leader male urine. To do this we used the average female distance to the leader male across all distance samples from the 2 month post-takeover period. In cases of infanticide, only samples collected prior to infant death were included. Further, females were only included in this analysis if we had at least 2 focal samples (or 6 distance samples) during this time period (the mean number of distance samples per female was 34.2). The mean distance to the leader male was compared between females that produced swellings during the 2 months post-takeover (N = 8) and those that did not swell (N=7).

Deceptive fertility as a counterstrategy to infanticide

Finally, we examined the effect of deceptive fertility indicators on infant survival. We tested how the presence of female sexual swellings and mating with the new male, both

separately and together, influenced the percent of infants surviving the takeover. This analysis used data from 27 females for which we had sufficient data documenting sexual swellings (at least 2 per month following their takeovers).

Statistical analyses

All statistical analyses were conducted using RStudio (Version 0.94.110). Due to the qualitative differences between takeovers, all cases (i.e., all females in all takeovers) were included separately. We acknowledge that this creates the problem of pseudo-replication in our data analyses and this should be considered when interpreting the results. Exact Poisson tests were used to compare the rate of postpartum swelling in the first and second months following takeover with the rate across all months outside of takeovers. All further analyses used either Welch's two sample t-test for comparing means or Fisher's exact tests for comparing proportions.

Results

Effect of takeover on the timing of postpartum sexual swellings

The first postpartum swellings are disproportionately more likely to occur in the first ($N = 22$) and second ($N = 10$) months following takeovers compared to the background rate outside of takeovers (0.96 females/month; exact Poisson tests: $p < 0.001$ for both months, Figure 5.1). Further, there was a trend towards females beginning to swell earlier on average (412.4 ± 166.1 days) following takeovers compared to the population mean of postpartum amenorrhea (481.7 ± 147.2 days) ($t(49.4) = -1.90$, $p = 0.06$, Figure 5.2). When females with infants that were beyond the theoretical age at which infanticide is a risk (1.26 yrs, Beehner & Bergman, 2008) are removed from the group of females that undergo a takeover (leaving $N = 22$, postpartum period: 395.3 ± 180.7 days), this difference becomes significant ($t(38.1) = -4.53$, $p < 0.001$).

Are these swellings deceptive?

Despite the early return to outward fertility, we found no difference in mean IBI length between females following takeovers (883.1 ± 242.5 days) and the population mean (900.1 ± 232.0 days) ($t(42.7) = 0.27, p = 0.78$). Assuming that pregnancy length is approximately 183 days (see Chapter Two), this indicates females after takeovers took significantly longer to become pregnant (411.5 ± 241.9 days) than is observed in the population mean (241.9 ± 185.4) ($t(38.5) = -2.86, p = 0.007$) (Figure 5.3). Further, when fE levels in the swelling females were examined, we found only one of 12 females had a single fE sample (21.03 ng/g) in the periovulatory range (21.45 ± 4.95 ng/g, see Chapter 2). All other females had fE levels that were higher than the population mean for lactating samples and fell between follicular and luteal phase means from normal ovarian cycles, never reaching periovulatory levels (Table 5.1; Figure 5.4, panels a and b). Moreover, although the temporal pattern for the ‘deceptive’ sexual swellings is abnormal compared to a normal ovarian cycle, females do reach peak swelling and do not outwardly look different from cycling females (Figure 5.4, panels c and d).

Exploring mechanism

The male proximity data indicate that females that produced sexual swelling were, on average, closer to the new leader male in the first 2 months following a takeover compared to females that did not swell ($t(6.1) = 3.62, p = 0.01$) (Figure 5.5).

Deceptive fertility as a counterstrategy to infanticide

We find that both swelling and mating with the new male increase the chances of infant survival. Infants of females that produced swellings had a higher chance of surviving than did infants of females that did not swell (Fisher’s Exact test: $p = 0.002$; Figure 5.6). Similarly, infants of females that mated with the new male had a higher chance of surviving than did

infants of females that did not mate (Fisher's Exact test: $p = 0.006$, Figure 5.6). In fact, infants of females that produced swellings, mated with the new male, or did both, all survived. In contrast, 75% of infants of infants that did not swell or mate were killed (Fisher's Exact test: $p < 0.001$, Figure 5.7)

Discussion

This study presents the first physiological evidence of deceptive swellings as a counterstrategy to infanticide with evidence of fitness advantage. We found that lactating female geladas disproportionally swell immediately after experiencing a takeover and that this outward "return to fertility" is accelerated compared to normal. However, this acceleration does not result in a shortening of their entire interbirth interval, nor do the swellings coincide with normal cycling levels of fE. In other words, there is no evidence that these swellings are honestly indicating fertility under these circumstances. Further, although not conclusive, our proximity data hint at male urinary estrogens as a potential mechanism for these deceptive swellings. Finally, we illustrated that deceptive fertility can be used as a successful counterstrategy to infanticide, at least in geladas.

Outside of geladas, deceptive swellings have only been recorded as a counterstrategy to infanticide in one other species, hamadryas baboons (Colmenares & Gomendio, 1988; Zinner & Deschner, 2000). Perhaps this is because deceptive fertility, in general, would only benefit females and be selected for in species in which (1) female production of indicators of fertility is flexible and not strictly limited to the periovulatory period as in many rodent species (Hrdy, 1979), and (2) males are not able to detect ovulation (e.g., Fuertbauer, Heistermann, Schuelke, & Ostner, 2011).

Alternatively, the lack of evidence in other species could be due to inability to conclusively illustrate that the sexual swellings are “deceptive”. To do this there must be evidence that the swellings occur outside of ovulation. As we discussed in Chapter Two, demonstrating ovulation is a daunting task in wild populations, but conclusively demonstrating its *absence* is even more difficult. Failure to detect ovulation could always be the result of inadequate sampling. Here, our hormone data are not adequate to conclusively illustrate the absence of ovulation, but it is clear that these females are not conceiving when they produce sexual swellings immediately following takeovers. This is sufficient evidence that these females are producing sexual swellings outside of fertile periods.

This raises the question of mechanism. Although we were not able to directly measure female exposure to male estrogens, it is clear that the lactating females producing swellings following takeovers were found closer to the new leader male than were females that did not swell. This pattern may have arisen because females position themselves close to the new male to increase exposure to male pheromones. The exposure could result in two pathways leading to increase estrogen level in the females themselves. First, it could result in the stimulation of the females’ own endogenous estrogens, raising it to a level that would produce the sexual swellings, but not enough to stimulate ovulation (and suppress lactation). Alternatively, this mechanism may be more similar to that in mice, where the male estrogens act directly on the female reproductive tissues (Guzzo et al., 2012). Further testing of grooming time and rates of copulations with the new leader may give a better estimate, at this time our focal samples do not provide sufficient sample sizes for this comparison.

There are several caveats that need to be addressed. The largest caveat in this study (as in Chapter Two) is that we cannot say with certainty that ovulation did not occur in the females

following takeovers. Progesterone levels would be required to illustrate that the formation of the corpus luteum did not occur – the one true indicator that ovulation actually took place. Again, we have had difficulty finding a sensitive antibody for progesterone, but plan to assay all samples for progesterone in the future. Another caveat is the lack of social data beyond copulations and distance to the leader male. It is plausible that other social factors are at play here, either in determining which females could (or would need to) produce swellings as a counterstrategy to infanticide or providing clues towards alternative successful strategies such as defensive alliances (as are seen in chacma baboons; e.g., Palombit et al., 2000). To address such possibilities, future work should include data on female dominance rank, aggression, and affiliative behaviors such as grooming. Our sample sizes here prevented us from looking at these variables in this study, but these variables will be necessary to complete our understanding of reproductive strategies in female geladas. Increasing sample size would also allow for more informative statistical analyses such as mixed models that would be able to include all variables at once in place of separate bivariate analyses. Despite these caveats, the results of this study are striking. Takeovers can clearly influence the reproductive physiology and behavior of lactating females. The resultant sexual swellings appear to be deceptive in nature and conclusively confer a fitness advantage to the females.

Table 5.1. Summary of fE results.

Female stage	Mean fE (ng/g) \pm SD	N	Mean samples per female
Swelling following takeover	6.90 \pm 2.94	12	5.2
Normal ovarian cycles			
Perioovulatory period	21.45 \pm 4.95	7	3.7
Follicular phase	12.30 \pm 1.45	3	13.0
Peak	36.90 \pm 7.41	7	--
Luteal phase	5.49 \pm 1.46	3	10.0
Lactation			
Lactation	2.62 \pm 1.52	59	7.2

Figure 5.1

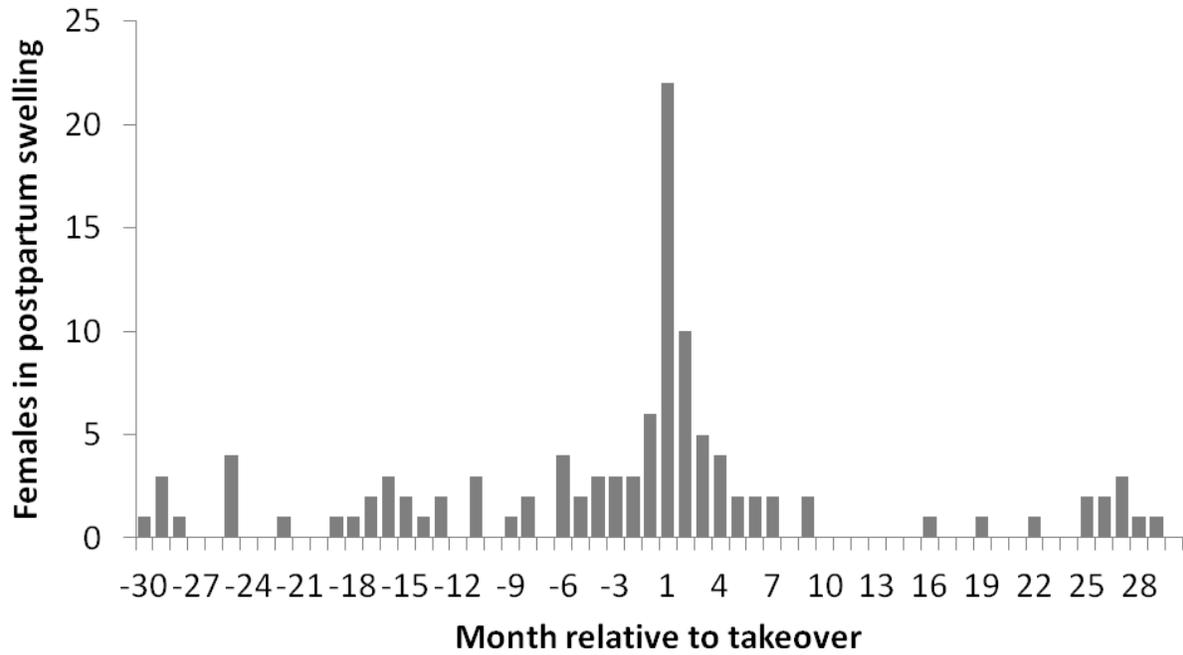


Figure 5.1. Number of females found in their first postpartum swelling in the months relative to takeover. Note that this graph is a limited view of this pattern, postpartum swellings occurred up to 49 months prior to and 68 months following the closest takeover in a pattern similar to the outside portions of this graph (never more than 3 females in any one month).

Figure 5.2

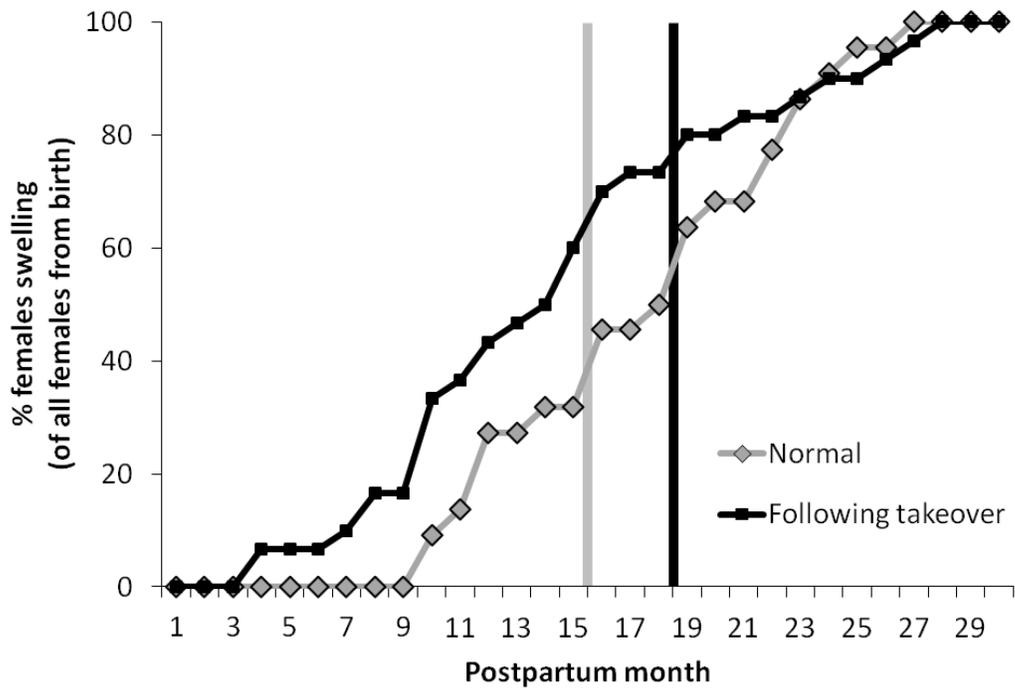


Figure 5.2. Percent of females swelling relative to birth following takeover and under normal circumstances. The vertical gray bar indicates the theoretical point when infanticide should no longer be adaptive for the male (Beehner and Bergman, 2008). The vertical black bar indicates the population mean for the first postpartum swelling.

Figure 5.3

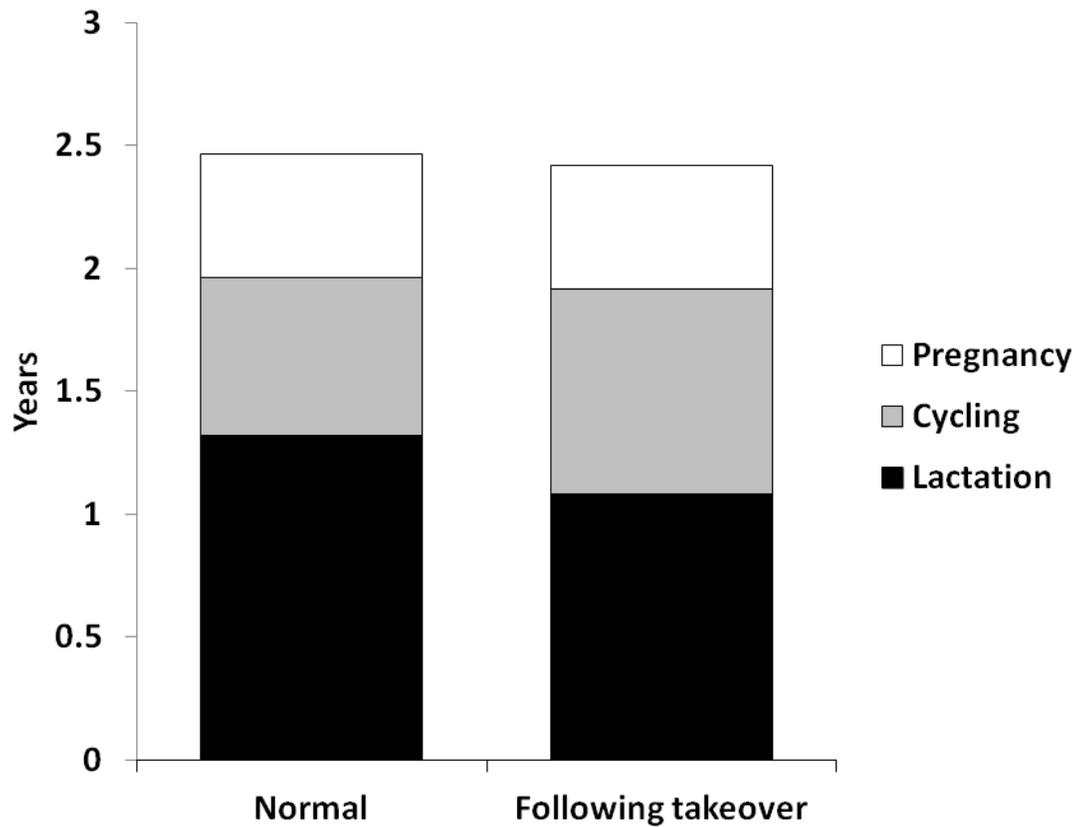


Figure 5.3. Comparing the IBI stages between the population mean and females following takeovers. Compared to the population means for reproductive data, lactating females after male takeovers resume signs of outward fertility much earlier (black), but take longer to conceive (gray). As a result, they do not have shorter interbirth intervals.

Figure 5.4

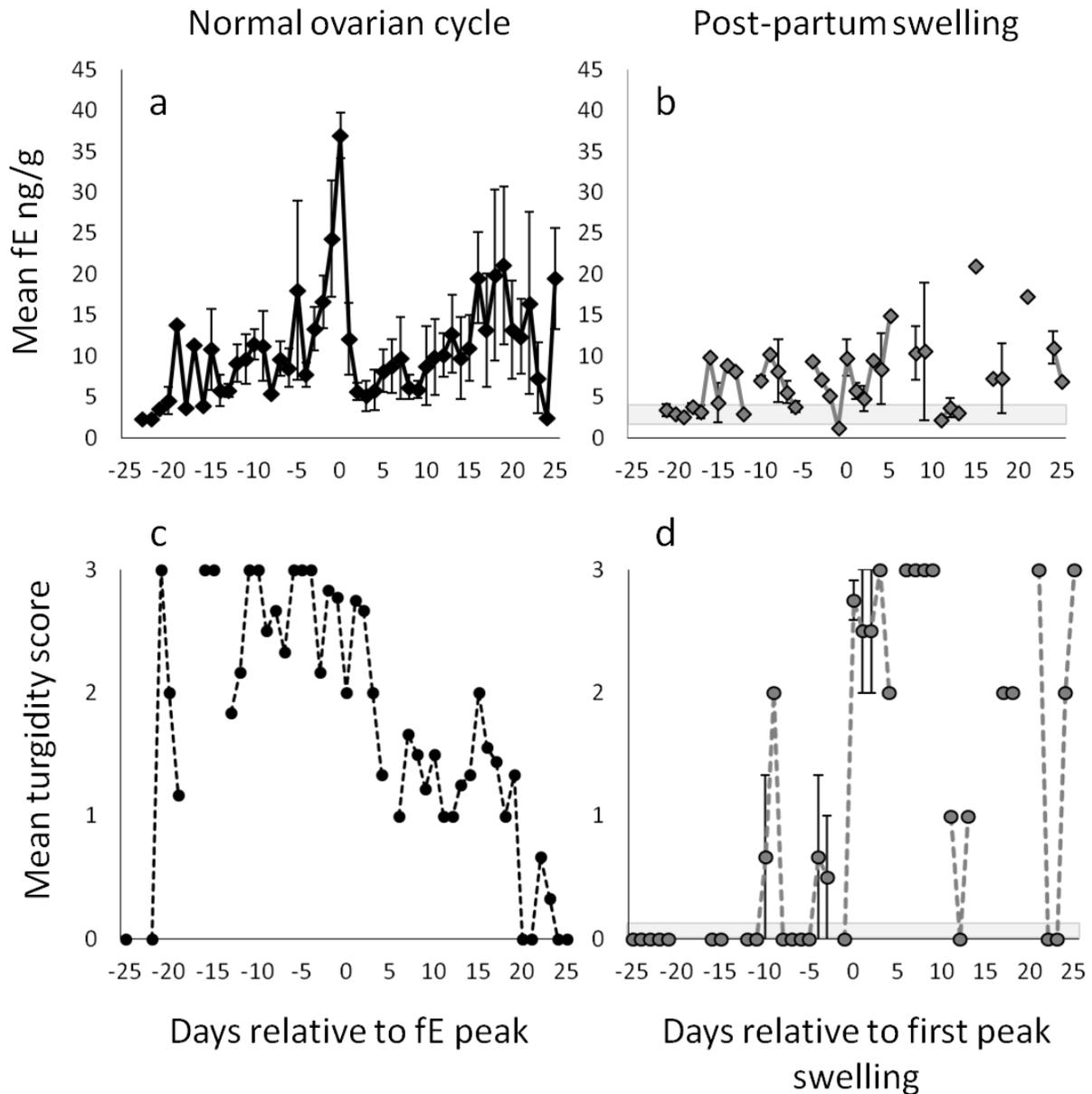


Figure 5.4. Comparing hormones and swellings in normal ovarian cycles and swellings following takeovers. After male takeovers, lactating females do not appear to produce regular estrogen cycles (panels a,b). Although these females do produce sexual swellings of the same turgidity found in normal cycling females, the temporal pattern is abnormal (panels c, d). The horizontal gray bars in panels b and d indicate the mean \pm SD for normal lactating females' fE and turgidity scores, respectively.

Figure 5.5

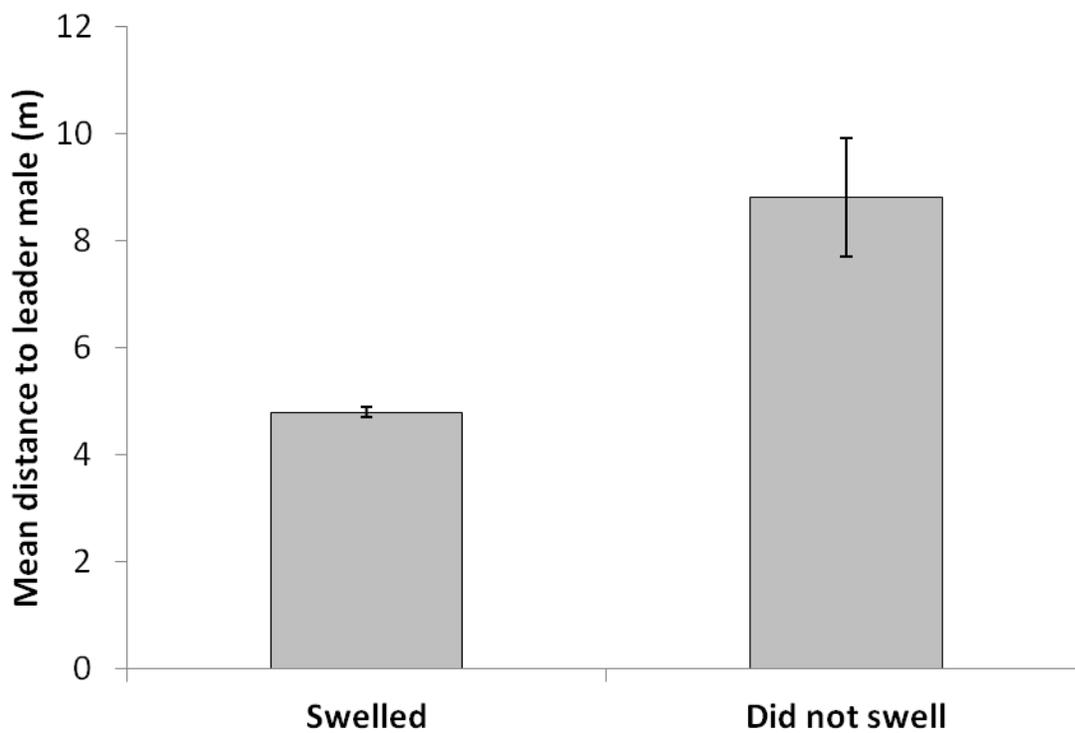


Figure 5.5. Distance to leader male in females that swelled following takeovers and those that did not swell. Lactating females that exhibit post-partum sexual swellings were found in closer proximity to the new leader male than lactating females that did not exhibit such swellings.

Figure 5.6



Figure 5.6. Infanticide outcomes relative to swelling and mating. Infants were more likely to be killed if their mother did not produce sexual swellings post-takeover. Likewise, infants were more likely to be killed if their mother did not mate with the new leader male following a takeover.

Figure 5.7

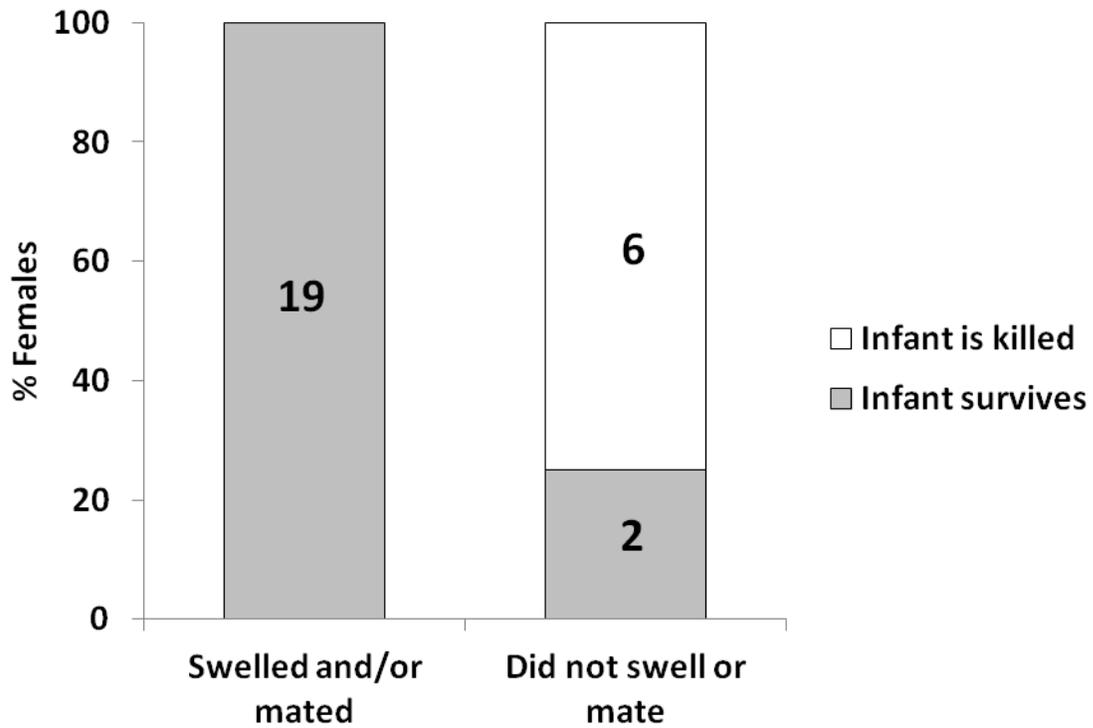


Figure 5.7. Infanticide outcomes relative to deceptive fertility indicators Infants are more likely to be killed if their mother did not swell or mate with the new male post-takeover.

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Chapter Six

Conclusion

This study contains the first results on the functional significance of changes in physiology, sexual swellings, and reproductive behavior of wild female geladas following male takeovers. Physiological profiles revealed that females perceive their own infanticide risk, spontaneously abort pregnancies, and produce deceptive fertility in response to male takeovers. This is the first study to conclusively illustrate the existence of a Bruce effect in a wild population as well as the first physiological evidence of deceptive swellings in lactating females. Moreover, we provide the first evidence that the Bruce effect and deceptive swellings can function as counterstrategies to infanticide. Together, these results comprise the most complete study to date of the function and underlying mechanisms (i.e., hormones and behavior) of female reproductive strategies in primates. Here, I provide a brief synthesis of our results in the context of directions for future research.

Answers to our questions

Our first major goal was to establish baseline patterns for female gelada reproduction in terms of: (1) broad patterns of life history stages that would allow us to investigate the function of any reproductive strategies and, (2), specific profiles of hormones, behavior and sexual swellings across the reproductive stages (i.e., ovarian cycles, pregnancy, and lactation) for

identifying potential mechanisms underlying reproductive strategies. In Chapter Two, we used fecal estrogen metabolites, demographic data, and sexual swellings to establish the most accurate estimates for life history stages including pregnancy, postpartum amenorrhea, and interbirth interval for this species. Our hormonal, behavioral, and sexual swelling profiles allowed us to identify two common counterstrategies to infanticide in our study population.

Our second major goal was to investigate infanticide risk and female perception of infanticide risk. In Chapter Three, we found that demographic factors can mediate infanticide risk for individual females and, following takeovers, female physiological stress response reflects this differential risk. More importantly, we found that infant survival is linked to higher physiological stress response. In spite of the widely accepted assumption that the acute stress response is adaptive, there is very little evidence that directly links the acute stress response to actual fitness outcomes (Breuner, Patterson, & Hahn, 2008). Our unique finding is evidence that an acute stress response can be beneficial to individual fitness, and is the first study of this kind for wild primates.

Our last major goal was to investigate female counterstrategies to infanticide in pregnant and lactating female geladas. We first found unambiguous physiological evidence of spontaneous miscarriages in geladas and illustrated a fitness advantage to females that employed this strategy (Chapter Four). Even though this is thought to be a relatively expensive counterstrategy to infanticide, the majority of pregnant females utilized this strategy in response to takeovers. Then, in Chapter Five, we found demographic and physiological evidence of deceptive fertility (both sexual swellings and behavior) following takeovers in lactating female geladas. Finally, we demonstrated that deceptive fertility is a 100% effective counterstrategy to infanticide in geladas.

These exciting findings notwithstanding, our studies have several limitations that need to be addressed. They have also, as all good research does, elucidated an entirely new set of questions. Below I will go through a number of limitations of this work and future directions inspired by it.

Overall limitations and future directions

The largest caveat is that we lack data on progesterone levels in females during normal reproductive events as well as following takeovers. Although we have worked with several antibodies, we have not been able to find one that is sensitive enough to pick up the differences between the follicular and luteal phases of the ovarian cycle. Therefore we are unable to conclusively illustrate that females have ovulated or, in the case of deceptive swellings, that they did not ovulate. Progesterone data are essential to complete our description of reproductive parameters as well as to confirm that the post-takeover swellings did, in fact, occur outside of ovulation. Identifying an antibody that would serve this function is our first priority for future work.

The behavioral data were also lacking in sample sizes to provide more information on the social context for the reproductive strategies. Despite the availability of hundreds of focal samples, we were not able to examine grooming, aggression, temporal patterns of proximity, or detailed sexual behavior. In some cases the focal samples were not frequent enough in the post-takeover period to be representative of female patterns, in other cases the focal samples simply did not include any social time (which is essential for grooming or fine-grained proximity data). Follow-up data on post-takeover behavior might be most interesting in the continued investigation into the mechanisms behind the counterstrategies to infanticide. If, as the mice data suggest (Guzzo, Jheon, Imtiaz, & Decatanzaro, 2012), males are excreting estrogens that can

enter the female via dermal absorption, grooming data and temporal patterns of proximity or contact could suggest a mechanism for the Bruce effect (given that estrogen can disrupt pregnancy) and deceptive swellings. Moreover, if females must actively remain near the leader male for this exposure, the risk associated with proximity to the potentially infanticidal male could explain the heightened stress response in females whose infants survive the takeovers. Although the analysis could not be completed with the current data set, combining demographic risk, hormones (both fE and fGCs), behavior, and swelling data on females into one complete statistical analysis would allow for a complete view of how these factors together result in individual female's infanticide outcomes.

This raises one of the more interesting future directions. If not the Bruce effect or deceptive fertility, what do females do in response to the threat of infanticide? Anecdotally, the two females that we observed to give birth following a takeover (i.e., that did not abort their pregnancies) yielded a survival rate of 50%. One female gave birth one month following the takeover and her infant was killed. The other female gave birth past the 2 month post-takeover period of infanticide risk. Did this prevent her infant from being killed? It is possible, but this particular takeover was also overrun with additional male followers. For much of the first year following the takeover this unit had at least 5 males, 2 of which were remaining from prior to the takeover (the deposed leader and a follower that had come in with him). The infant may have been protected by the father or perhaps the distraction of increased male numbers kept the new leader too busy. In cases like this, identifying which factor, or which combination of factors, prevented the infant death is too difficult. Once we are able to collect enough data on enough takeovers to get a handle on the different social factors that might be important, we could pursue this line of study. In terms of alternatives to deceptive fertility, there were two females whose

infants survived that did not produce sexual swellings and were not observed to mate. Here, again, more detailed behavioral data would potentially provide a clue as to what else these females might have been doing to protect their infants.

The second follow-up question is: If we have shown that both the Bruce effect and deceptive fertility confer such clear fitness advantages to females, why don't all females use them in all takeovers? Although we can only speculate at this point, we suggest that the answer will eventually be found among the mechanisms, female factors, and environmental cues.

In terms of mechanisms, the delicate balance of hormones that define different reproductive stages may make females more or less able to use physiological strategies like the Bruce effect and deceptive swellings at certain times. However, as discussed above and in previous chapters, we currently do not have the data on behavior or the methods for collecting physiological data to fully explore these.

Alternatively, there could be factors specific to the female or environmental cues related to the takeover, that affect the female's ability or incentive to use a counterstrategy. These same factors might also influence the overall risk of infanticide. For example, in Chapter Three, we examined how infant age, female parity (as a proxy for female age), group size, and father presence influence infanticide risk. We examined infant age and female parity as factors related to the female that could mediate her own risk, and perhaps, what counterstrategy she could use. Group size and father presence, on the other hand, are environmental cues that we investigated.

Beyond these, there are many other factors that may influence infanticide risk and female counterstrategies following takeovers. In Table 6.1, I have provided a list of potential factors that could be included beyond those from Chapter Three. Among them are additional female factors such as individual history of takeovers and infanticides that could explain the development of

any counterstrategies across a female's lifetime. Additionally, a female's exact age and her age at first birth (generally a good predictor of lifetime reproductive success) could provide clearer information for cost/benefit analyses concerning where she is in her potential reproductive lifetime. Other environmental cues may be important as well. For example, meteorological patterns such as rain and temperature should correlate with food availability and thus with female health. Exploring such ecological factors could clarify shorter term costs and benefits for females. For example, if food is abundant and the current infant is very likely to survive, the cost of infanticide would be very high. In contrast, if food is scarce and the infant is in poor health, the relative cost of infanticide decreases. Due to our lack of knowledge regarding the potential mechanisms, female factors, and environmental cues of female counterstrategies, we cannot make definitive conclusions for now. We have, however, identified a number of avenues to pursue in the future.

Table 6.1. Summary of factors that are proposed to influence infanticide risk and female counterstrategies.

Factors examined in Chapter Three	
Female Factors	Female parity Infant age
Environmental cues	Group size (number of females) Father presence
Factors to be considered in future work	
Female Factors	Age at takeover Dominance rank History of takeovers History of infanticides Age at first birth Quality of relationship with infant's father
Environmental cues	Meteorological patterns Number of males Ratio of males to females in the unit

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